Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum

Dissertation

Submitted to the

NIMS UNIVERSITY RAJASTHAN, JAIPUR

In partial fulfilment of the requirement for the award of degree of

MASTERS OF SCIENCE

IN

NUTRITION & DIETETICS

2024-2026

By-

DRISHTI LALWANI

Enrolment No: 145634

Under the supervision of:

Dr. Damini Soni

Assistant Professor

Department of Nutrition and Dietetics

NIMS COLLEGE OF ALLIED AND HEALTHCARE SCIENCES

NIMS UNIVERSITY RAJASTHAN, JAIPUR

Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum

A dissertation submitted in partial fulfilment of the award of

M.Sc. Nutrition and Dietetics

(Master of Science in Nutrition & Dietetics)

Degree to

NIMS UNIVERSITY RAJASTHAN, JAIPUR

By

Drishti Lalwani

Enrolment No: 145634

Under the guidance of

Guide:

Dr. Damini Soni

Assistant Professor

Department of Nutrition and Dietetics

Nims College of Allied And Healthcare Sciences

Jaipur, Rajasthan

Certificate

This is to certify that the dissertation on **“**Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum” was done by Drishti Lalwani (Enrolment No. 145634) under my supervision. I am satisfied with the work presented by the candidate towards the partial fulfilment of the Master of Science in Nutrition & Dietetics.

GUIDE:

Dr. Damini Soni

Assistant Professor

Department of Nutrition & Dietetics

Nims College of Allied And Healthcare Sciences

Jaipur, Rajasthan

Declaration by student

I solemnly declare that the dissertation entitled **"Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum"** is an original research work approved by Institutional Research Cell carried out by me under the guidance of Dr. Damini Soni, Department of Nutrition & Dietetics, Nims Institute of Nutrition and Public Health. This dissertation is submitted to NIMS University, Rajasthan , Jaipur to fulfill the requirement for the Master of Science (MSc) award in Nutrition and Dietetics.

I further declare that to the best of my knowledge, this dissertation has not been submitted to another university or institution in India to award any degree or diploma.

Place: Jaipur
Drishti Lalwani

Date:

Department of Nutrition and Dietetics

Nims College of Allied And Healthcare Sciences

NIMS University, Rajasthan, Jaipur

Enrolment No: 141574

Certificate of Research Supervisor

This is to certify that the dissertation “Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum” is submitted by Drishti Lalwani, postgraduate student, to the NIMS University, Rajasthan , Jaipur , for the degree of Master of Science in Nutrition and Dietetics. It is a novel piece of work carried out by the student under my supervision and attesting to the originality of the work that has not been submitted for the award of other degree/diploma of the same Institute where the work was carried out, or to any other Institution.

It is further certified that the student has fulfilled all the requisite conditions/requirements laid down by the University. Accordingly, this dissertation is eloquently fit for the award of the degree of Master of Science in Nutrition and Dietetics.

Place: Jaipur
Dr. Damini Soni

Date:
Assistant Professor

Department of Nutrition and Dietetics

Nims College of Allied And Healthcare Sciences

NIMS UNIVERSITY RAJASTHAN, JAIPUR.

Acknowledgment

I thank God Almighty for giving me the strength, good health, and resources to complete this research successfully. I express my sincere gratitude to my guide, Dr. Damini Soni, Assistant Professor, Department of Nutrition and Dietetics, NIMS Institute of Nutrition and Public Health, NIMS University, Rajasthan, Jaipur, for her guidance and support during this study. I also thank the faculty and staff of the department for their assistance throughout the course of this work.

I express my deepest and most sincere love and gratitude to my parents, whose unconditional support, unwavering faith, have been the foundation of everything I have achieved. Their constant encouragement, emotional strength, and endless patience have seen me through every demanding phase of my research, and I must say that their faith in me has always been my biggest motivation.

I take this opportunity to express my sincere appreciation for the invaluable support provided to me by my cousin brother, Mr. Garv Jivnani, who dedicated his time, effort, and enthusiasm towards helping me prepare the practical aspects of my ice cream formulations. His contribution to my research work has been invaluable towards the successful completion of my research.

I extend my warm and heartfelt thanks to my cousin sister, Ms. Yashi Jivnani, for her creative expertise and meticulous efforts in designing the nutritional label and packaging of the developed product. Her aesthetic

sensibilities have ensured that the packaging design is not only visually appealing but also fully compliant with the FSSAI regulatory standards.

Lastly, I would like to acknowledge with great respect all the various researchers, scientists, and scholars who, through their published work, have formed the academic basis for this work. The various contributions to the field of functional food science, plant-based nutrition, prebiotic science, and vegan food product development have greatly informed, guided, and enriched the academic content of this dissertation. I am grateful to all of them for their pioneering work in this field.

I am grateful to one and all who, in one way or another, have contributed to this research work.

Place: Jaipur,
Drishti Lalwani

Date:
Enrolment No: 145634

Department of Nutrition and Dietetics

Nims Institute of Allied And Healthcare Sciences

NIMS UNIVERSITY, RAJASTHAN, JAIPUR

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ABSTRACT	
I.	INTRODUCTION	
II.	REVIEW OF LITERATURE	
III.	METHODOLOGY	
IV.	RESULTS AND DISCUSSION	

V.	**SUMMARY AND CONCLUSION**	
	REFERENCES	
	APPENDIX	

LIST OF TABLES

Table No.	Title	Page no.
1.1		
1.2		
1.3		
1.4		
1.5		
3.1		
3.2		
3.3		
4.1		

4.2		
4.3		
4.4		
4.5		
4.6		

LIST OF FIGURES

Figure No.	Description	Page No.
1.1		
1.2		
1.3		
1.4		
1.5		
1.6		
1.7		
1.8		
1.9		
1.10		
1.11		
1.12		
3.1		
3.2		
3.3		
3.4		
3.5		
3.6		

3.7		
3.8		
3.9		
3.10		
3.11		
4.1		
4.2		
4.3		
4.4		
4.5		
4.6		
4.7		
4.8		

LIST OF ABBREVIATIONS

ABBREVATIONS	EXPANSION
AAA	Formulation Variation T1 (Gond Katira 35 g, Cashews 70 g)
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) — antioxidant assay
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
BBB	Formulation Variation T2 — Most Acceptable (Gond Katira 45 g, Cashews 85 g)
BMJ	British Medical Journal
CAGR	Compound Annual Growth Rate
CCC	Formulation Variation T3 (Gond Katira 55 g, Cashews 55 g)
CFU	Colony Forming Units
CHO	Carbohydrate
DDD	Control Formulation
DPPH	2,2-Diphenyl-1-picrylhydrazyl — free radical scavenging assay
EAAI	Essential Amino Acid Index
FAO	Food and Agriculture Organization of the United Nations
FDC	Food Data Central (USDA)
FOS	Fructooligosaccharides
FRAP	Ferric Reducing Antioxidant Power
FSSAI	Food Safety and Standards Authority of India
GAE	Gallic Acid Equivalents

GOS	Galactooligosaccharides
HDPE	High-Density Polyethylene
ICMR	Indian Council of Medical Research
IFCT	Indian Food Composition Table
IMARC	International Market Analysis Research and Consulting Group
ISAPP	International Scientific Association for Probiotics and Prebiotics
ISO	International Organisation for Standardisation
NABL	National Accreditation Board for Testing and Calibration Laboratories
NIMS	Nims Institute of Medical Sciences
NIN	National Institute of Nutrition
OBMS	Oat-Based Milk Substitute
ORAS	Opal Research and Analytical Services
PBMA	Plant-Based Milk Alternatives
PBMS	Plant-Based Milk Substitutes
RDA	Recommended Dietary Allowance
RSM	Response Surface Methodology
SD	Standard Deviation
SMP	Skimmed Milk Powder
SOP	Standard Operating Procedure
SPSS	Statistical Package for the Social Sciences
SVTL	SV Testing Laboratories Pvt. Ltd.
TEAC	Trolox Equivalent Antioxidant Capacity

TISTR	Thailand Institute of Scientific and Technological Research
TPC	Total Phenolic Content
TSS	Total Soluble Solids
UPF	Ultra-Processed Food
USD	United States Dollar
USDA	United States Department of Agriculture
WPC	Whey Protein Concentrate
XOS	Xylooligosaccharides

ABSTRACT

Background

The increasing market demand for plant-based functional foods and the increased awareness of gut health have created a major market window for vegan prebiotic high protein-containing frozen desserts.

Objectives

The present study was initiated with an aim to formulate and characterize a vegan prebiotic high protein ice cream using germinated amaranth milk-based protein, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, FOS/inulin, almond butter, coconut milk, dark chocolate, cocoa powder, cashews, and vanilla extract.

Methods

The study was conducted at NIMS University, Jaipur, Rajasthan and was carried out in six systematic phases. Four different formulations were developed, one control formulation and three experimental formulations, in which the amounts of gond katira and cashews were varied. Sensory evaluation was performed using a 9-point hedonic scale method with 15 semi-trained panelists. The highest-scoring formulation was selected for further nutritional and physicochemical analysis through a NABL accredited laboratory.

Results

The formulation containing 45g gond katira and 85g cashews was selected as the best formulation with an overall acceptability score of 6.1 ± 1.7. NABL accredited nutritional analysis confirmed that the product contains 6.87g protein, 290.86 Kcal total energy, 7.90g fat, 51.97g carbohydrates, 1.19g crude fibre, 29.84% moisture, a melting rate of 30 minutes, total phenolic content of 195 mg GAE, DPPH radical scavenging activity of 24.90%, off white colour, and an overrun of 78% per 100g. The cost of production was calculated at ₹424.07 per 500g unit. The product was packed in a food-grade HDPE container, labelled as per FSSAI Labelling and Display Regulation, 2020, and named Greenz Ice Cream under the brand name Nutrii Mantra.

Conclusion

The developed formulation is nutritionally superior, organoleptically acceptable, microbiologically safe, and commercially feasible, with strong potential in the Indian market of functional and plant-based frozen desserts.

KEY WORDS

Vegan ice cream, prebiotic, high protein, germinated amaranth milk, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, functional food, plant-based, gut health, antioxidant, FSSAI, Nutrii Mantra.

CHAPTER - I

INTRODUCTION

INTRODUCTION

1.1. Ice Cream: Importance, Composition, and Market Evolution

1.2. Health Concerns Associated with Conventional Ice Cream

1.3. Emergence of Vegan and Plant-Based Ice Cream

1.4. Functional and Symbiotic Ice Cream

1.5. Plant based milk substitutes (PPBS)

1.6. Nutritional and Functional Potential of Selected Plant-Based Ingredients

1.6.1 Amaranth

1.6.2 Makhana (*Euryale ferox*)

1.6.3 Jackfruit Seed Powder

1.6.4 Gond Katira

1.6.5 Monk fruit sweetener

1.1 ICE CREAM - Importance, Composition, and Market Evolution

Ice cream is really special when it comes to food that is made in factories. It is something that people enjoy eating because it tastes good it is a tradition that many cultures share. It is also a way for scientists to make food that is good for us. People of all ages and backgrounds from around the world eat ice cream. Over time ice cream has changed from a mix of frozen milk, sugar and flavourings to a very complicated food system that people have carefully designed.

When we look at ice cream from a point of view it is made up of many different parts like ice, fat, air and a special liquid that is all mixed together in a way that is not very stable. Ice cream has ice crystals, globules that are stuck together air bubbles and a special liquid part that is not frozen all of which are in a delicate balance, with each other. [1,2] The way these phases are made up. Like how big the ice crystals are, how much the fat is mixed up how much air is, in the mix. How the air bubbles are arranged. Is what makes the product feel and taste a certain way. This is what makes the product creamy, smooth and nice to eat. It is

also what makes the flavour come out and what makes the product not melt fast. [3]

Getting a handle on the structure of ice cream is really important for people who make ice cream and for people who want to create new ice cream products. Ice cream science and product development depend on this. People have made some progress in learning about these tiny structures. They have used tools, like X-ray microtomography and cryo-scanning electron microscopy to do this. [4]

The ingredients used to make ice cream are many and work together. They include milk or plant-based alternatives sweeteners that make it sweet and affect how it freezes and feels. There are also emulsifiers, stabilizers that help keep it flavourings, colorants and some special additives that have health benefits. Sweeteners have an impact, on ice cream. They not make it sweet but also affect how it freezes how thick the liquid part is, and how it melts.[1] Stabilisers and emulsifiers work together to help control the way acts and to keep air bubbles stable. This has an effect, on the texture of something and how well it melts. Stabilisers and emulsifiers are really important because they help make sure that the texture and the way something melts are just right.[5] Consumer expectations for ice cream have really changed. People now want ice cream that not tastes good but also provides some nutritional value. They prefer products made with clean-label ingredients. They are also looking for ice cream that offers health benefits and is environmentally sustainable. This shift has changed research priorities. Now researchers focus on creating plant-based and functional ice cream formulations. The ice cream industry is moving towards making products that're good for people and the planet. People want to enjoy ice cream while also making eco-friendly choices. The trend is moving away from indulging in ice cream for pleasure. Instead, consumers want ice cream that offers more. They want products that align with their values and support their well-being. The ice cream industry is adapting to these changing consumer expectations. It is focusing on creating products that

meet these demands. Plant-based and functional formulations are, at the forefront of this change. These types of ice cream products are becoming more popular. They offer a combination of taste, nutrition and sustainability that consumers are looking for.[6] People pay attention to the information about the food they eat. The way this information is shown on the package also matters. When it is easy to understand people are more likely to buy food. This is what studies have found out about the labels on food packages the simple nutrition symbols, on the front.[7]

The ice cream market around the world is getting bigger and bigger. It was worth seventy-five billion dollars in the year two thousand twenty three. The ice cream market is expected to keep growing at a rate of five-point two eight percent every year. This means the ice cream market will be worth one hundred nineteen billion dollars by the year two thousand thirty-two.

The part of the ice cream market that is really taking off is the ice cream segment. This includes ice cream that is made from plants has sugar and has probiotics and prebiotics. The functional ice cream segment is growing fast. It was worth two hundred forty million dollars in the year two thousand twenty-four. The functional ice cream segment will be worth four hundred forty million dollars, by the year two thousand thirty-two. It is growing at a rate of seven-point three four percent every year.[8] People are changing the way they eat. This change is happening faster. This is because of a thing: more people are paying attention to what they eat and trying to be healthy more people are finding out they are lactose intolerant more people are becoming vegan or flexitarian and more people want to eat in a way that is good, for the planet. [9]

1.2 HEALTH CONCERNS ASSOCIATED WITH CONVENTIONAL ICE CREAM

Conventional ice cream made from dairy has a nutrition profile that is being looked at closely by nutrition scientists and public health experts

over the world. This type of ice cream has a lot of calories because it has a lot of sugars and saturated fats. These ingredients are linked to problems, like being overweight obesity, type 2 diabetes, heart disease and insulin resistance when eaten much. They can also lead to metabolic disorders. A lot of research has shown that eating a lot of fat is connected to a risk of many health issues. These issues include not heart and metabolic diseases but also cancer, neurological disorders, chronic respiratory problems and gastrointestinal issues.[10]

Food companies make ice cream in factories. This type of ice cream is called -processed food. The NOVA framework says that ultra-processed foods are made from ingredients that people do not usually use when they cook at home. These ingredients include things that make food taste good look nice and feel smooth. They also include things that make food taste sweet. The NOVA framework is a way to group foods, into categories. Ice cream falls into the ultra-processed foods category because it is made with these kinds of ingredients.[11] A review published in 2023 found that eating foods with UPF raised the risk of obesity by 32 percent. It also showed that eating foods increased the risk of type 2 diabetes by 37 percent, high blood pressure by 32 percent and high triglycerides by 47 percent. The study looked at UPF and its effects, on health. UPF stands for -processed food. These foods are often linked to health problems. The review was an analysis of many studies.[12] A big study that looked at 45 studies published in the BMJ in 2024 found out that people who get more Ultra Violet Radiation exposure are more likely to die from anything get heart disease, type 2 diabetes feel depressed and have bad sleep. The study really showed that more Ultra Violet Radiation exposure is not good for people. It can cause a lot of health problems, like disease and type 2 diabetes and also affect people's sleep and mood leading to depressive outcomes and adverse sleep-related outcomes. Ultra Violet Radiation exposure is an issue. [13] People did a study to see how eating a lot of ultra-processed food affects the risk of adult obesity. This study looked at other studies and found a clear link between eating ultra-processed food and the risk of obesity, in adults.

This means that eating -processed food can really increase the risk of obesity. So it is an idea to eat food that is not processed as much. This is another reason why food companies should make food that's not as processed.[14] The quality of the food we eat is really important when it comes to how ultra-processed foods affect our health. Eating quality food can actually reduce the bad effects that ultra-processed foods have on our bodies. The quality of our diet is like a middleman, between eating a lot of -processed foods and how healthy we are. If we eat a lot of -processed foods but also eat a lot of good quality foods then the bad effects of the ultra-processed foods are not as strong.[15] These findings are really interesting. They have made companies change their products. They are taking out sugars and putting in natural ones instead. They are also taking out fats and using fats from plants. Additionally, they are adding things like fibre, prebiotics and special nutrients to make their products better, for people. [6] When we look at sweetener and seed incorporation in ice cream, we find that it works for both dairy and vegan ice cream. These substitutions can make the ice cream better in terms of quality. They can also increase the antioxidant activity. The good thing is that this does not affect how the ice cream tastes to people who eat it. Sweetener and seed incorporation in ice cream is a good way to improve ice cream. Sweetener and seed incorporation, in dairy ice cream and vegan ice cream can make these products better.[16]

Lactose intolerance is a problem, for people who love eating ice cream. It stops many from enjoying ice cream. 65% Of adults worldwide have this issue. In East Asia it affects 70 to 100% of people. In South Asia, West Africa and the Middle East the numbers range from 60 to 80%.[17] This problem comes from the fact that the body does not have enough of a helper called intestinal lactase-phlorizin hydrolase. This helper is necessary to break down lactose; a type of sugar found in milk and other foods into things like glucose and galactose in the small intestine. When lactose is not broken down it moves to the intestine. There tiny living things like bacteria feed on the lactose and make gases, like carbon dioxide and hydrogen. These gases cause the body to react with

symptoms like feeling bloated having gas getting stomach cramps and having diarrhoea. [18] Some people are lactose intolerant. Others have a milk protein allergy. This allergy is usually to casein and whey protein. It is a problem for 2 to 3 percent of people in the world. People, with this milk protein allergy need to stop eating dairy products There are also people who choose not to eat dairy products because they are vegan. Then there is the issue of the environment. Dairy farming uses a lot of land and water. It also makes greenhouse gases. So, we really need to have dairy-free alternatives. Milk protein allergies and vegan people and the environment are all reasons why we need these dairy- alternatives.[19]

1.3 EMERGENCE OF VEGAN AND PLANT-BASED ICE CREAM

Veganism defined as the practice of abstaining from all animal derived products including meat, dairy, eggs, and honey has undergone a profound transformation from a niche dietary philosophy into a mainstream lifestyle movement with wide-reaching implications for the global food industry. The motivations driving this shift are multifaceted ethical concerns regarding animal welfare and factory farming, environmental sustainability considerations particularly the documented contribution of animal agriculture to greenhouse gas emissions, land degradation, and water consumption and evidence-based health motivations. [20] Among pseudo cereal based plant-based meal solutions quinoa is really special. It has all the protein we need has a mix of amino acids and is rich, in minerals and fibre.[21] A comprehensive review of life cycle assessments by Scarborough et al. published in Nature Food (2023) demonstrated, using dietary data from 55,504 UK participants, that vegan diets had greenhouse gas emissions representing only 25.1% of those of high meat-eaters, land use of 25.1%, water use of 46.4%, and eutrophication impact of 27.0% — a striking demonstration of the environmental dividend of plant-based dietary choices. [22] These findings confirm what we already thought. Going for a plant-based diet can really

help greenhouse gas emissions. In fact, it can reduce them by much, as 49 to 70 percent worldwide. [23] People are really interested in learning about the food we eat and how it affects our lives. The study of plant-based diets is a deal now. It looks at how vegetarianism and veganism impact our society and our health. Vegetarianism and veganism are taken seriously and are now important areas of study with big effects, on our world.[24] Plant-based dietary patterns have been recognised as simultaneously beneficial for human health and planetary sustainability, offering co-benefits across reduced chronic disease burden, lower greenhouse gas emissions, and decreased resource use.[25]

The plant-based milk alternatives (PBMA) market has been one of the most commercially successful categories to emerge from the vegan movement, with global market value projected to reach USD 30.8 billion by 2031. [26] Innovative vegan ice cream formulations using legume-fermented bases such as tempeh milk combined with moringa leaf flour have demonstrated the breadth of plant-based frozen dessert innovation emerging from research institutions.[27] Enrichment of vegan ice cream with fermented hazelnut cake has been shown to improve nutritional density and bioactive content, expanding available formulation strategies.[28] Consumer research confirms that while taste and texture remain primary purchase drivers, ethical values, environmental sustainability, and health motivations are increasingly influential, particularly among younger demographics who have grown up in a period of heightened climate awareness. [29] Cross-cultural survey research further confirms that taste quality, ingredient transparency, and ethical values are the principal drivers of vegan ice cream purchase decisions.[30] In India the vegan ice cream market was worth about thirty million dollars in 2024. It is expected to grow to around fifty million dollars by 2033. This is because people in India really like plant based and dairy food. They have been eating this kind of food for a long time. The vegan ice cream market in India is changing fast. New flavours are being made all the time. Companies are using Indian things like jaggery and dates to make these new flavours. They are also using chia seeds and

millet. Fruits that are grown in India are being used too. The vegan ice cream market, in India is very exciting.[31]

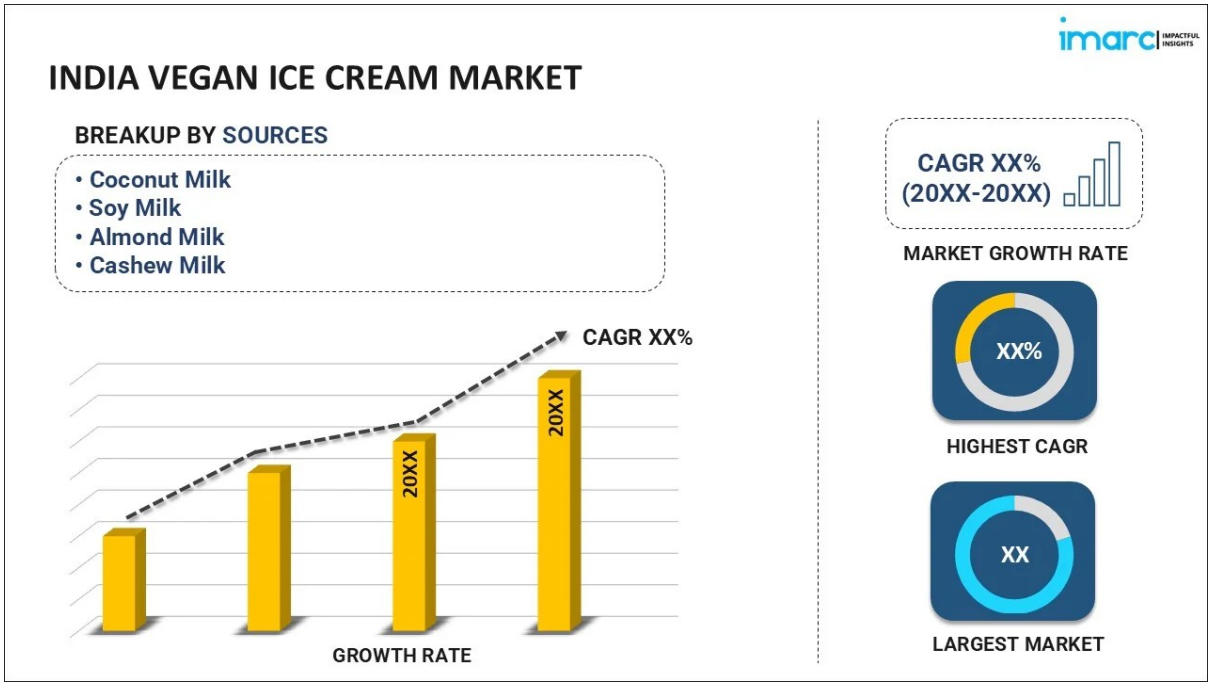


Figure 1.1: Global ice cream market growth and projected CAGR Market Research Future, 2024

Vegan ice cream is a dessert made from plants. It tries to be like ice cream but uses no animal products. It is usually made with a base from plant milk. This base comes from things, like cereals nuts legumes seeds or pseudocereals. It is mixed with plant oils or fats sweeteners stabilisers and flavouring.[26] Nutritionally, well-formulated vegan ice cream is inherently cholesterol-free and may provide meaningful quantities of dietary fibre, unsaturated fatty acids, plant polyphenols, and antioxidants, which are largely absent in dairy counterparts. [18] However making plant-based ice cream is still tricky. This is because it does not have dairy proteins, like casein micelles and whey fractions. These proteins help with mixing making foam and combining fat in ice cream. So plant-based ice cream makers must choose hydrocolloids, emulsifiers and plant proteins carefully. They need to pick the ones to get a similar texture to regular ice cream. [32] People are making vegan ice cream with plant-based milks, like kidney bean milk. They are also adding probiotics to the vegan ice cream. The vegan ice cream made with red kidney bean

milk and probiotics works well. Has good qualities.[33] Hemp drink-based ice cream formulated with plant proteins and guar gum has similarly demonstrated that targeted selection of hydrocolloids and proteins can produce acceptable physical properties in diverse plant-based systems.[34] Research has confirmed that vegan ice cream formulated with appropriate probiotic cultures and prebiotic fibres can achieve viable probiotic counts of 6–9 log CFU/g, satisfying international benchmarks for probiotic food health claims. [35] A comprehensive review of dairy-free alternatives for frozen dessert applications has outlined key formulation strategies, stabiliser systems, and technological challenges specific to this product category.[36]

1.4 FUNCTIONAL AND PREBIOTIC ICE CREAM

The concept of functional food — food that provides measurable health benefits extending beyond basic nutritional sustenance — has become a central paradigm in global food product development. Ice cream is particularly well-positioned as a functional food vehicle, combining exceptionally broad consumer acceptance with a physicochemical structure — the aerated oil-in-water emulsion with a substantial continuous aqueous phase — that is capable of hosting and protecting diverse bioactive compounds through processing, freezing, and storage. A systematic review by Genovese et al. (2022) on functional ingredients in ice cream confirmed that their inclusion consistently improved nutritional density, modified textural characteristics, and — in the case of prebiotic and probiotic additions — produced demonstrable gut health outcomes in experimental models.[37] Ingredients successfully incorporated into functional ice cream include dietary fibres, inulin and FOS, plant polyphenols, bioactive peptides, phytosterols, carotenoids, and vitamins, alongside whole functional ingredients such as algae, seeds, and novel plant flours. [6]

The International Scientific Association for Probiotics and Prebiotics or ISAPP says that Prebiotics are things that the tiny living things inside our

body use to help our body be healthy. Prebiotics are used by these living things to make our body healthier. The ISAPP is talking about Prebiotics. How they work with the tiny living things, in our body to give us health benefits.[38] A food ingredient is a prebiotic if it does three things. First the prebiotic has to be able to withstand the acid in the stomach and the enzymes that break down food in the body. The prebiotic also has to be able to be broken down by the bacteria in the colon. The prebiotic has to help the good bacteria in the body grow and be more active which makes the body healthier. The prebiotic does this by helping the bacteria that are already, in the body like the beneficial microorganisms to work better and make the body stronger. This is what makes the prebiotic a prebiotic. [39] A review of inulin-type fructans published in Advances in Nutrition in 2022 summed up evidence from trials. It showed that inulin-type fructans have prebiotic effects on gut bacteria. They help increase the growth of bacteria like Bifidobacterium, Lactobacillus and Faecalibacterium prausnitzii. This leads to health benefits. These benefits include an intestinal barrier, better bowel movements and improved insulin sensitivity. Inulin-type fructans also help triglycerides and improve cholesterol levels. They increase the absorption of calcium and magnesium in the body. Additionally, they help people feel fuller for longer. The evidence supports the benefits of inulin-type fructans, for health. [40] Clinical intervention trials have further confirmed that inulin-type fructan supplementation produces measurable beneficial shifts in gut microbiota composition, with consistent increases in bifidobacterial abundance and improvements in metabolic health markers.[41]

The mix of bad bacteria in our gut is getting worse because of the way we eat today. We eat a lot of processed foods and not enough fibre. This has led to an increase in gut problems worldwide. These problems are connected to long-term diseases like type 2 diabetes, heart disease being bowel disease. A review in the Journal of Clinical Medicine in 2025 looked at studies that showed prebiotics, probiotics and a mix of both, called symbiotic can help in ways. They help good bacteria grow make short-chain fatty acids that feed the cells in our colon and help our

system. They also make our gut stronger. Reduce inflammation by affecting a specific pathway, in our body.[42] Incorporating prebiotic fibres into widely consumed food products such as ice cream therefore constitutes a strategically viable and evidence-based public health approach. [43] Inulin has similarly been demonstrated to support the growth of vegan yoghurt cultures in pea protein-based frozen dessert systems, simultaneously enhancing textural properties and prebiotic activity.[44]

The role of prebiotics in ice cream is really important. Prebiotics like inulin are very useful. Inulin makes the ice cream mix thicker stops ice crystals from growing when you store the ice cream and helps it melt slowly. It also makes the ice cream lighter and fluffier because it can form a gel. This means you can use fat in the ice cream and it will still taste good. For example, some researchers found out that prebiotic fibers, inulin and apple fiber help the good bacteria in the ice cream stay alive when it goes through your body. They did an experiment in a lab to see what happens to the bacteria when you eat the ice cream. They found out that when you mix prebiotics and probiotics together the good bacteria can survive and even thrive. Other things like gum and guar gum are also very useful in ice cream made from plants. They help keep the ice cream smooth and stop the ice crystals from growing. They can also be used as food for the bacteria, in your body. This is a good thing because it can help keep you healthy. [43] Psyllium mucilage has similarly been evaluated as a natural stabiliser in vegan yogurt ice cream, with findings confirming meaningful improvements in physical firmness and sensory texture.[45]

1.5 PLANT-BASED MILK SUBSTITUTES (PBMS)

Plant-based milk substitutes (PBMS) are aqueous extracts of plant materials that are processed, homogenized, and stabilized to resemble animal milk in appearance, texture, and—where possible—nutritional profile. Formally, they constitute colloidal systems in which a continuous

aqueous phase serves as the dispersing medium for starch granules, protein aggregates, lipid droplets, and residual plant cell wall fragments. The practical motivation for PBMS extends beyond veganism: the combination of lactose intolerance affecting 65% of adults globally, a growing pool of milk protein allergy sufferers, and the well-documented environmental cost of dairy milk production—in terms of greenhouse gas emissions, land use, and freshwater consumption—has created a structural and durable demand for plant-based alternatives.[20] Life cycle assessments have consistently demonstrated that all categories of PBMS generate substantially fewer greenhouse gas emissions, require less agricultural land and freshwater, and produce lower levels of aquatic eutrophication compared to bovine dairy milk production.[46]

Plant Based Milk Substitutes are made from lots of kinds of plants. We get them from cereals like rice, oat, spelt, kamut, corn and rye. We also get them from nuts like almond, coconut, walnut, hazelnut, pistachio and tiger nut. Then there are pseudocereals like quinoa and amaranth. Plant Based Milk Substitutes also come from legumes, which include soy, pea, cowpea, peanut and kidney bean. We even get them from seeds, like flax, sesame, hemp, sunflower and pumpkin.[20] The nutritional characteristics of Plant Based Milk Substitutes vary a lot depending on the plant they come from and how they are made. Plant Based Milk Substitutes like the ones made from soy are pretty close to cow milk when it comes to the amount of protein and the quality of acids they have. On the hand Plant Based Milk Substitutes made from almonds have very little protein in them. The ones made from oats are special because they have something called beta-glucan which helps lower the cholesterol in our blood. Plant Based Milk Substitutes made from hemp are sources of essential fatty acids and they have just the right amount of omega-6 and omega-3. One big problem with Plant Based Milk Substitutes compared to cow milk is that they do not have as much of some important nutrients like calcium, vitamin D, vitamin B12, riboflavin and an amino acid called lysine that we find in the ones made from grains. We can fix these problems by adding these nutrients to the Plant Based Milk Substitutes on purpose and, by

mixing plant sources together to get a more complete mix of amino acids. [47]

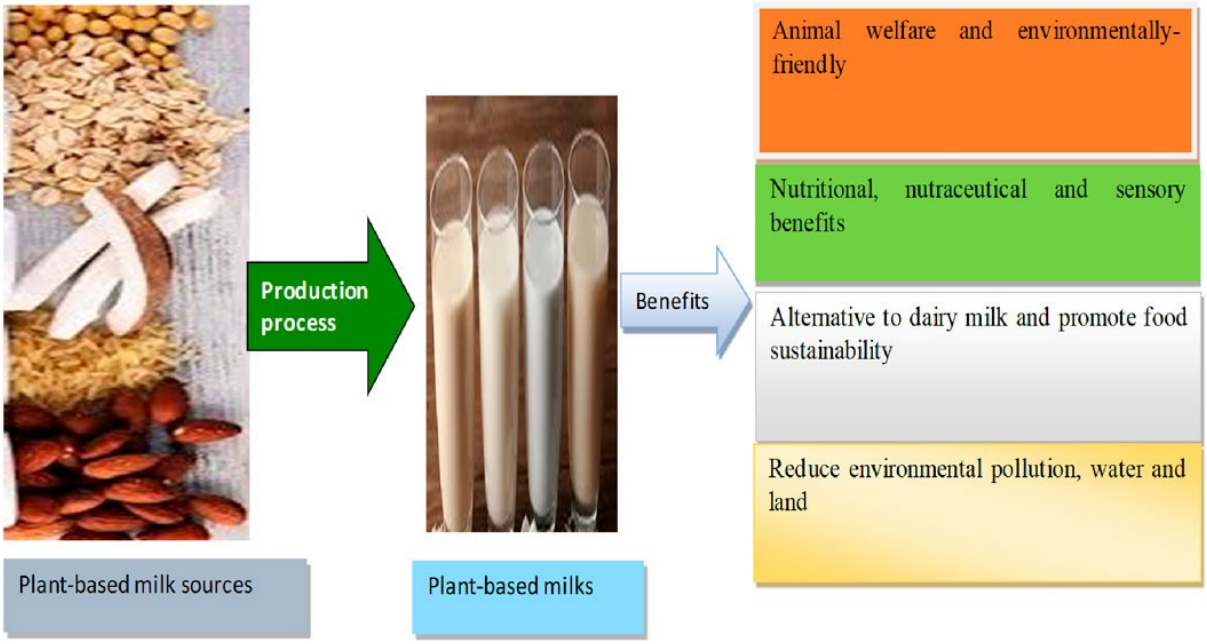


Figure 1.2 : Sources of plant-based milk substitutes (PBMS).

Source: Irondi EA et al., Frontiers in Food Science and Technology, 2025

When you look at Peanut Butter Made Snacks or PBMS you see that they have a lot of things in them. These things include acids and flavonoids and phytosterols and bioactive peptides and dietary fiber. All of these things, in PBMS work to help with antioxidant and antihypertensive and anti-inflammatory and antidiabetic properties. This is what the experimental studies have found about PBMS. [20] Advanced processing technologies, including high-pressure homogenization, ultrasonication, enzymatic hydrolysis, and fermentation, have been shown to substantially improve the stability, bioavailability of nutrients, and sensory acceptability of PBMS, significantly expanding the range of plant sources amenable to high-quality commercial application. [38] The global population, projected to reach 10 billion by 2050 and increasingly urbanized, demands food systems that are both nutritionally adequate and environmentally sustainable. In this context, PBMS and the functional food products derived from them—including plant-based ice

cream—represent a convergent solution to the dual challenges of human health optimization and planetary resource stewardship. [20]

1.6 NUTRITIONAL AND FUNCTIONAL POTENTIAL OF SELECTED PLANT BASED INGREDIENTS

1.6.1 AMARANTH (Amaranthus spp.)

Amaranth (Amaranthus spp.) is a genus of approximately 60 species within the family Amaranthaceae, classified as a pseudocereal on the basis of its seed morphology, culinary usage, and nutritional profile despite its non-membership of the grass family Poaceae. [48] The three species historically cultivated for grain production are Amaranthus cruentus (red amaranth), Amaranthus caudatus (love-lies-bleeding), and Amaranthus hypochondriacus (Prince-of-Wales feather), all of which were central crops for pre-Columbian civilizations in Mesoamerica before European colonization.[48] Today people are really interested in amaranth again. The Food and Agriculture Organization of the United Nations also known as the FAO thinks that amaranth is one of the promising crops to help with food problems around the world. This is because amaranth is very good, for you it does not have. It can grow well in tough conditions. Amaranth can handle not having water it can handle salty soil and it can even handle very high temperatures. These things are very important now because of climate change. Because we do not have as much water as we need.[49]

The amount of protein in amaranth grain is around 15.4 to 16 percent when it is dry. This is really good compared to grains like wheat which has 13.5 to 14.5 percent protein, maize with 10.6 to 13.8 percent, barley with 10 to 14.9 percent and oat with 12.4 to 12.9 percent. Amaranth protein is very special. It has a lot of lysine which's an important part of protein. Amaranth has 55 to 65 milligrams of lysine per gram of protein. This is two times more than wheat and three times more than maize. This makes amaranth a great addition to diets that are based on plants

because lysine is often the part that's missing. Amaranth has a lot of amino acids around 47.6 grams per 100 grams of protein. This is a lot more than what the Food and Agriculture Organization and the World Health Organization say we need to be healthy. Amaranth also has a lot of cysteine and methionine which're important for our bodies. These are often hard to find in proteins from beans. This means amaranth is a match, for both grains and beans when we mix them together.[48]

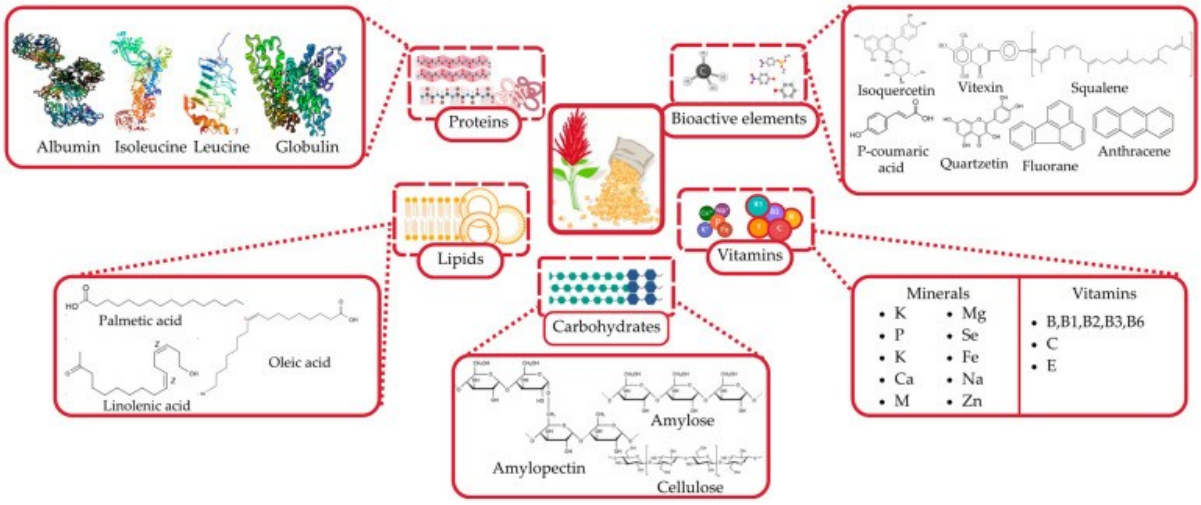


Figure 1.3 : Nutritional and phytochemical composition of amaranth grain.

Source: Toimbayeva D et al., Foods, 2025.

Amaranth grain has a lot of protein in the perisperm areas. This is different from grains, where the protein is mostly in the endosperm. The way protein is spread out in amaranth grain makes it very good for you. It has a high protein efficiency ratio, which's around 1.5 to 2.0 and a biological value of about 75. This is as good as or even better than other grains.

The fat in amaranth grain is also very good for you. It makes up about 6 to 9 percent of the grains weight. Some types of amaranth grain can have much as 19 percent fat. The good thing about this fat is that it has a lot of fatty acids, which are good for your health. Most of the fatty acids in amaranth grain are acid, which is about 50 to 62 percent. Then there is acid, which is about 16 to 26 percent.

Amaranth grain also has something called squalene, which's very good for you. It helps keep your liver healthy boosts your system and protects your skin. Squalene is also an antioxidant, which means it helps keep you from getting sick. It is very rare to find a food that has much squalene. Amaranth grain also has something called tocopherols which're like vitamins that help keep the fat in the grain from going bad.

Amaranth grain has a lot of good things in it too. It has flavonoids, like quercetin and kaempferol which're like special helpers that keep you healthy. It also has acids, like ferulic acid and gallic acid, which are good for you. Then there are betaxanthins and betacyanins like amaranthine, which give the grain its color. Amaranth grain also has carotenoids, like lutein and zeaxanthin which're good for your eyes.

All these special helpers in amaranth grain can do a lot of things for you. They can help lower your blood pressure stop tumors from growing and keep your blood sugar under control. They can also help boost your system and keep you from getting sick. Some studies have even shown that peptides, from grain can help keep you from getting hypertension, diabetes and dyslipidemia.[49]

Table 1.1: Proximate Composition and Mineral Content of Amaranth Grain (per 100 g dry weight) [18,21]

Proximate Component	Range / Value	Mineral / Antinutrient	Range / Value
Protein (%)	15.4 – 16.0	Magnesium (mg/kg)	2300 – 3360
Fat (%)	5.6 – 9.7	Calcium (mg/kg)	1300 – 2850
Ash (%)	2.5 – 3.2	Sodium (mg/kg)	160 – 480
Dietary Fibre (%)	3.1 – 6.6	Iron (mg/kg)	72 – 174
Carbohydrates (%)	60.0 – 69.0	Zinc (mg/kg)	36.2 – 40.0
Energy (kcal/100 g)	370 – 380	Phytic acid (g/kg)	2.9 – 7.9
Squalene (% of oil)	6.0 – 8.0	Saponins (mg/kg)	0.9 – 4.91
Total Essential AA (g/100g protein)	47.6	Trypsin inhibitors	Trace amounts

Although amaranth's nutritional profile is outstanding, the grain does contain antinutritional factors — tannins, phytates (2.9–7.9 g/kg), saponins (0.9–4.91 mg/kg), oxalates, and nitrates (concentrated in leaves, not grain) — that can reduce micronutrient bioavailability and impart undesirable bitter or astringent sensory notes.[48] However, these antinutrients are highly responsive to standard food processing interventions, particularly germination. Research has demonstrated that 48-hour germination at 26°C increases protein availability by 8%, total energy by 11%, and linoleic acid content by 10%, while reducing resistant starch by approximately 70%, indicating substantially improved starch digestibility and protein bioavailability. [50]

Combined soaking and germination treatments have been shown to produce significant reductions of 32.30% and 29.57% in tannin and phytic acid contents respectively, and to simultaneously increase crude protein, crude fibre, total phenolic content, and antioxidant activity. [51] Fermentation with lactic acid bacteria provides complementary benefits: it has been shown to reduce phytic acid from 11.06 to 1.00 mg/g and tannins from 4.92 to 2.05 mg/g, while increasing fat, protein, and ash content by 3%, 22%, and 14%, respectively. [48] Innovative non-thermal processing technologies including cold plasma, high hydrostatic pressure, pulsed electric fields, and ultrasonic processing have also emerged as efficient strategies for antinutrient reduction in amaranth. [49]

Amaranth has been successfully incorporated into a remarkably diverse range of food product categories. In baking, partial substitution of wheat flour at 15–30% significantly improves the protein content, mineral density, and overall nutritional value of bread, with sensory evaluation revealing acceptable consumer acceptability. Gluten-free amaranth flour at 25% incorporation in cookie formulations improves essential amino acid content, mineral concentration, and physical quality attributes. Amaranth-based sports beverages have demonstrated enhanced cycling performance in comparative trials against commercial sports drinks, attributable to their superior amino acid and mineral profile. [48]

Amaranth has further been incorporated into pasta, noodles, extruded snacks, baby food preparations, gluten-free clinical nutrition products, and functional beverages, consistently demonstrating nutritional enhancement. [49] A comprehensive review of nutraceuticals and bioactive compounds in quinoa and amaranth has further documented their shared richness in phenolic acids, flavonoids, and antioxidant peptides, reinforcing the functional food potential of both pseudocereals. [52] Emerging research has highlighted the promise of amaranth protein encapsulation for bioactive compound delivery, and the development of amaranth-based edible films and coatings for sustainable food packaging. In the context of the present research, germinated amaranth milk — produced by soaking, germination, grinding, filtering, and pasteurisation of amaranth grain — serves as the principal plant-based milk base, leveraging the substantially enhanced digestibility, reduced antinutritional factor load, improved bioavailability, and superior bioactive compound content that germination confers relative to ungerminated grain milk. [49]

Table 1.2: Key Bioactive Compounds in Amaranth Grain and Their Documented Health Activities [18,21]

Compound Class	Specific Compounds	Documented Health Activities
Phenolic acids	Vanillic, gallic, ferulic, caffeic, syringic acid	Anti-cancer, anti-obesity, anti-diabetic, anti-inflammatory, antioxidant, anti-bacterial
Flavonoids	Quercetin, kaempferol, rutin, vitexin, isovitexin	Antioxidant, antifungal, anti-carcinogenic, anti-inflammatory, hepatoprotective, neuroprotective, cardioprotective
Betacyanins	Amaranthine	Antioxidant, antimicrobial, cytotoxic

Compound Class	Specific Compounds	Documented Health Activities
Carotenoids	Lutein, zeaxanthin, β-carotene	Antioxidant, anti-diabetic, anti-inflammatory, anti-cancer, anti-osteoporosis, vision-protective
Tocopherols	α, β, γ, δ-tocopherols	Antioxidant, cardioprotective, anti-cancer, anti-diabetic, anti-obesity
Squalene	Triterpenoid squalene	Antioxidant, hepatoprotective, immunostimulatory, skin-protective

1.7 MAKHANA (Euryale ferox Salisb.)

Makhana (Euryale ferox Salisb.), vernacularly known as foxnut or gorgon nut, is an aquatic plant of the family Nymphaeaceae that produces nutritionally dense seeds within submerged fruits. It grows in slow-moving freshwater bodies—ponds, lakes, and flooded depressions—primarily across the Indian subcontinent and Eastern China, with Bihar state in India accounting for approximately 80–90% of national production. Makhana has been. Eaten in India and China for more than three thousand years. It is very important in medicine, traditional Chinese medicine and religious rituals. In Ayurveda Makhana is thought of as a tasting, heavy and strength building substance. It is given to people who're weak have diarrhoea, urinary problems and reproductive health issues.

In Chinese medicine Makhana is valued because it is good for the spleen and kidneys and has astringent properties. To make Makhana seeds into a snack they are heated to a temperature until they pop. This makes the seeds big and white. People like to eat them. Makhana seeds are also used in traditional dishes like kheer and raita. If we look at the nutrients

in 100 grams of popped Makhana seeds we can see that they have 11.16% protein, 75.04% carbohydrates and a little bit of fat. Makhana seeds also have some calcium, phosphorus, magnesium, iron, zinc and potassium which're all good for us. Makhana is a food to eat because it has a lot of nutrients and it is good, for our health.[53] The protein quality of makhana is exceptional: it shows an Essential Amino Acid Index (EAAI) of 89–93%, which surpasses many common cereal grains, and possesses an arginine + lysine to proline ratio of 4.74–7.6, a parameter associated with cardiovascular health support. [54] Its mineral profile is characterized by low sodium and high potassium content, contributing to blood pressure regulation support, and very low monounsaturated fat content, making it appropriate for blood sugar management. [53]

The antioxidant content of makhana varies notably with processing: raw seeds have higher phenolic content and DPPH radical scavenging activity compared to popped seeds, as high-temperature processing degrades heat-sensitive phenolic compounds. The principal free phenolics in raw makhana seeds include catechin (1.806 mg/100 g) and gallic acid (1.336 mg/100 g). [55]

Makhana is really good for you. It is not just because of what it contains. The things that makhana can do to help your body are very interesting. It helps keep your blood sugar levels in check which's great for people with diabetes. Makhana also helps you feel full so you do not eat much. This is because makhana has a lot of something called starch and it does not raise your blood sugar levels too quickly. Makhana is also very high in fiber, which's good for your intestines and helps you go to the bathroom regularly. Makhana has things in it called flavonoids and phenolic compounds like catechin and gallic acid. These things help keep your body safe, from damage. May even help keep your heart healthy and prevent cancer. Makhana is a thing to eat if you want to stay healthy.[56] The polyphenolic profile and antioxidant activity of edible aquatic plants more broadly have been characterised as sustainable sources of bioactive compounds, situating makhana within a wider category of nutritionally

valuable aquatic plant ingredients.[57] In traditional reproductive medicine, makhana consumption is associated with improved sperm count and quality, and TCM texts prescribe makhana-based preparations for the treatment of spermatorrhoea, attributing its reproductive benefits to moisture-balance modulation and hormonal effects. [58] The allergen-free status of makhana—it is free from the major eight food allergens—renders it particularly valuable for inclusion in allergen-free food product development. [59] Makhana has further been evaluated as a primary ingredient in formulated food products, with consumer acceptability trials confirming its sensory suitability and potential for diversified applications.[60] A systematic review of functional properties of makhana-based value-added products has documented consistent improvements in protein utilisation, antioxidant capacity, and textural characteristics when incorporated as a functional ingredient.[61]

1.8 JACKFRUIT SEED POWDER

Jackfruit, which comes from the Moraceae family is actually the fruit that trees produce all over the world. It grows in not-so-warm areas in South Asia, Southeast Asia, East Africa and some parts of Latin America.

India is the number one producer of jackfruit making around 1.8 million tonnes every year. In India jackfruit is mainly grown in states, like Assam, West Bengal, Uttar Pradesh, Maharashtra, Kerala, Tamil Nadu and Karnataka.[62] The fruit's anatomy includes an outer spiny rind, stringy inner rags, fleshy yellow arils (the edible bulbs), and numerous large seeds enclosed in pale fibrous coverings. While the arils attract widespread consumption, the seeds—comprising 10–15% of total fruit weight—are predominantly discarded as agricultural waste, with an estimated 90% of all seeds produced globally being wasted, despite their considerable nutritional and functional value. [63] This waste represents both a lost nutritional resource and a missed opportunity for value addition within a circular agricultural economy. [64] Quality evaluation of value-added products prepared from jackfruit seed flour has further

demonstrated favourable physicochemical and sensory characteristics, affirming the ingredient's broad culinary and functional applicability.[65]

The nutritional profile of jackfruit seeds is really good. Every 100 grams has 7 grams of protein. It has 1 gram of fat. It also has 1.5 grams of fiber.38 Grams of carbohydrates. It has 184 calories. Jackfruit seeds have a lot of minerals like potassium. About 246 milligrams phosphorus. 200 Milligrams magnesium , calcium 33 Milligrams, zinc and iron 1 Milligram. They also have vitamins like thiamine and riboflavin. These vitamins help with energy in our cells. Fight bad stuff. I am talking about jackfruit seeds and their nutrients. They have stuff that our body needs. Jackfruit seeds are really good for us. The minerals and vitamins, in jackfruit seeds are very important.[62] A 2025 comprehensive review confirmed that jackfruit seeds contain starch at 12.86–17.90 g/100 g and protein up to 18%, with dietary fibre of 1.56–3.92 g/100 g. [66]

The major protein jacalin—a unique tetrameric lectin found exclusively in jackfruit seeds—has been the subject of extensive immunological research for its capacity to activate T lymphocytes and modulate immune responses, with applications explored in HIV immune status assessment and cancer immunology. [62] The seeds of the jackfruit have a lot of things in them that can affect our bodies. These things are called compounds and they include catechin, epicatechin, ferulic acid, rutin, gallic acid and caffeic acid. All of these compounds together are what make the jackfruit seeds have an antioxidant capacity. This is what the tests, like DPPH, ABTS and FRAP assays have shown. [67] Lignans, isoflavones, and saponins present in the seeds have been attributed with anticancer, antiulcer, antihypertensive, and anti-aging properties in experimental models. [64] The seeds of the jackfruit have some good things going for them. They can help with inflammation. They have properties that fight off bad bacteria. The jackfruit seed also has things that're good for the microbes in our body. It is like a food for the microbes, in our stomach. This helps our body have good microbes and it helps our body make some important things it needs. [68] Jackfruit seeds also provide high magnesium

content, supporting blood pressure management and bone health through calcium absorption facilitation. [67]

Regarding functional food applications, jackfruit seed flour and powder demonstrate desirable technological properties including high water absorption capacity (WAC), oil-holding capacity (OHC), swelling index, and emulsification capacity, making them versatile functional ingredients across food product matrices. [64] A 2023 study confirmed that jackfruit seed powder enriches yogurt formulations with improved protein, mineral, and antioxidant content, demonstrating its practical utility as a natural nutrient supplement. [69] Processing of jackfruit seeds by autoclaving, boiling, or controlled drying at 50–60°C followed by grinding effectively reduces trypsin inhibitors and tannins while preserving protein quality. [37] The low cost, local availability, and inherent sustainability value of utilising otherwise-wasted jackfruit seeds in value-added functional food products — reducing agricultural waste while enhancing nutritional density — make jackfruit seed powder an ideal ingredient for the prebiotic plant-based ice cream formulation proposed in the present study. [63] The feasibility of jackfruit seed milk as a base for vegan ice cream has been demonstrated in prior studies, establishing a foundational precedent for its use in plant-based frozen dessert innovation.[70]

1.9 GOND KATIRA (TRAGACANTH GUM)

Gond Katira, known internationally as tragacanth gum, is the dried exudate obtained from incisions made in the stems and branches of Astragalus gummifer Labillardière and related Astragalus species of the family Fabaceae, native to the mountainous arid regions of the Middle East and Central Asia, including Iran, Turkey, Syria, and Afghanistan. [71] It is one of the oldest food-approved hydrocolloids in recorded history, with documented use in food, pharmaceutical, and cosmetic preparations spanning thousands of years. The dried exudate presents as pale, translucent, flattened ribbons or irregular flakes that are odorless and

tasteless in dry form; upon hydration, they absorb many times their dry weight in water, swelling into a characteristic soft, gelatinous, mucilaginous mass that is the foundation of their functional utility. [72] In homes and many Middle Eastern cultures people consider Gond Katira a must-have summer drink. They soak it in milk, water or a sweet drink called sherbet. This is because it helps to cool down the body keeps you supports digestion. In medicine Gond Katira is known as 'Sheetala' which means cooling. It is believed to help with 'Pitta dosha'. Doctors recommend it for problems like burning sensations, much thirst and issues with urine.

Gond Katira gum has two parts: tragacanthin and bassorin. Tragacanthin mixes with water. Helps to keep things stable and well mixed. Bassorin forms a gel- substance that makes the gum thick and helps it hold water. The gum is made up of sugars, like arabinose, galactose, xylose, fucose and galacturonic acid.[72] Nutritionally, per 100 g, Gond Katira provides approximately 70 kcal, 35 g carbohydrates, and 30 g dietary fibre — a composition that positions it as a low-calorie, high-fibre ingredient compatible with modern functional food formulation goals. [71] The gum also exhibits notable stability across a wide pH range (2–8) and temperature range, retaining its viscosity-enhancing and gel-forming properties in both acidic and alkaline food matrices—properties that are particularly advantageous in ice cream applications where the product must maintain stability across processing, freezing, and storage conditions. [73]

Gond Katira works well in frozen desserts. When you add it to the ice cream mix it makes the mixture thicker. This helps to slow down the formation of ice crystals as the mixture freezes gets harder and is stored. As a result you get an ice cream with a more even texture. The ice cream also feels creamy in your mouth. Doesn't melt as quickly. This is similar to fat dairy ice cream but without using artificial additives. This approach fits with the trend of using labels in product development. Gond Katiras properties make it ideal for use, in desserts. It helps to create a high-

quality ice cream that consumers will enjoy. The use of Gond Katira supports the creation of clean-label products.[72]

Its pharmacological applications in traditional systems of medicine include treatment of pharyngitis, diarrhea, gonorrhea, dysentery, and trachoma.[73] Gond Katira is resistant to hydrolysis by gastric acid—a property conferred by the bassorin fraction—and is subsequently degraded by anaerobic microflora in the colon, establishing the mechanistic basis for its prebiotic potential and its suitability as a carrier for colon-targeted drug delivery systems. [73] Recent research on chemically modified gum katira polysaccharides—including carboxymethylation, hydroxypropylation, and crosslinking modifications —has expanded the functional repertoire of this natural gum for applications in drug delivery, wound healing materials, and edible coating systems, demonstrating the broadening commercial relevance of this ancient natural ingredient. [73]

1.10 MONK FRUIT SWEETENER

The need to cut down on refined sugar intake is very important. This is because it is linked to health problems like obesity, diabetes and tooth decay. As a result many industries and people are shifting towards sweeteners that are low in calories.

At the time scientists are taking a closer look at artificial sweeteners. They want to know if these sweeteners are safe to consume and what effects they have on our bodies. A study by Suez et al. In Nature found that some artificial sweeteners like aspartame, sucralose and saccharin can change the balance of bacteria in our gut. This can lead to problems like glucose intolerance. Later studies on humans have also raised concerns about the long-term effects of these sweeteners, on our metabolism.[74] Some artificial sweeteners have been paradoxically linked to increased body weight, insulin resistance, and type 2 diabetes risk in epidemiological studies — an irony given their intended role as dietary aids for weight and blood glucose management. [75] This evidence has

created a compelling demand for natural sweeteners that are safe, zero-calorie, compatible with gut microbiota, and free from the safety concerns associated with synthetic alternatives. [76]

Monk fruit, also known as Siraitia grosvenorii Swingle is a round greenish-brown fruit. It comes from the Guangxi Zhuang Autonomous Region in China. This fruit is considered one of the natural sweeteners that are very sweet. In Chinese it is called 'Luo Han Guo'. The name comes from monks who grew it many years ago. The fruit belongs to the gourd family. People, in China have used fruit in traditional medicine for a long time. They use it for problems and digestive issues. Some people also think it helps them live longer. The fruit has good things in it. These include triterpenoids, flavonoids and vitamins. It even has proteins and oils that can evaporate. Because of these monk fruit is considered a food with many benefits. These benefits have been documented in studies.[75]

The monk fruit sweetness comes from compounds called mogrosides.These are made up of a base called mogrol and different amounts of glucose units attached. Mogroside V is the sweet part. Mogrosides are 100 to 250 times sweeter than sugar. Some are 250 to 400 times sweeter. The mogrosides are separated from the sugars in the fruit during processing. This results in a sweetener with zero calories from carbohydrates. It also has an index of zero. This makes monk fruit extract great for people with diabetes or insulin resistance. The US Food and Drug Administration said it is safe to use in 2010. It is okay to use in food and drink products. When using fruit sweetener it works best when it is not more than 25% of the product. If it is more, than that it can taste bitter and metallic. This is because of the mogrol base. It can make the product less enjoyable to eat or drink.[75]

Research by Kahler (2020) further demonstrated that natural sweeteners, including monk fruit, support the viability and metabolic activity of probiotic lactic acid bacteria, making them particularly suitable for use in symbiotic functional food formulations such as the prebiotic vegan ice cream proposed in the present study. [76]

The health benefits of fruit are really great and it is not just useful as a sweetener that has no calories. Monk fruit has properties that can help with a lot of things like stopping coughs helping people with asthma. It has antioxidants that can protect the liver and help keep the immune system working well. It can also help lower blood sugar. Might even help prevent cancer. A study done by Ban and others in the year 2020 looked at what happens when you put fruit extract in yogurt. They found out that it makes the yogurt have antioxidants, which is a good thing. The yogurt also had a texture and consistency and it was still good after it was fermented. This means that monk fruit can be used in a lot of products like dairy and non-dairy foods and it will still be good, for you.[77] People want to be healthy. They like natural sweeteners such as monk fruit. This is because monk fruit is good, for people who want to eat sugar take care of their blood sugar levels keep their gut healthy and only eat foods with simple ingredients. Monk fruit is a sweetener that meets all these needs.[78]

Significance of the Study

1. This research develops a functional vegan ice cream by incorporating germinated amaranth milk, providing a high-quality and easily digestible protein source that addresss the low protein content in most existing vegan ice creams.
2. Resistant starches from foxnut and jackfruit seeds are included as natural prebiotics to promote digestive health, while tragacanth gum enhances product stability and adds dietary fiber to support metabolic balance.
3. The formulation is designed to meet the nutritional needs of diverse groups, including vegans, lactose-intolerant individuals, athletes seeking protein-rich foods, and older adults requiring increased protein intake.
4. The addition of nuts and dates enriches the ice cream’s nutritional profile by supplying healthy fats, essential minerals, antioxidants, and natural low-glycemic sweetness.
5. Utilizing underutilized crops like foxnut and jackfruit seeds promotes sustainability by reducing food waste and encouraging the use of neglected agricultural resources, offering a unique and innovative product in the expanding plant-based food market.

AIM:

To formulate a prebiotic plant-based protein rich ice cream using amaranth milk, foxnut, jackfruit seed powder, tragacanth gum.

OBJECTIVES OF THE STUDY:

PRIMARY OBJECTIVE

- To standardize and develop a prebiotic vegan high-protein ice cream using germinated amaranth milk, foxnut, and tragacanth gum.

SECONDARY OBJECTIVE

- To conduct market survey for existing plant-based, prebiotic and high protein ice creams
- To prepare and standardize a prebiotic, plant-based high-protein ice cream.
- To analyze the physicochemical properties, proximate composition and antioxidant potential of the developed product.
- To conduct the sensory evaluation of the developed ice cream for taste, texture, and overall acceptability.
- To assess the cost and shelf life of developed product.
- To design packaging and a clear nutrition label that highlights the product’s benefits.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Devalekar and Udachan (2025) [79] aimed to create a healthy vegan ice cream. Coconut milk, date syrup, and fructooligosaccharide (FOS) were used to create it. Individuals seek out plant-based and low-sugar sweets, prompting the creation of this ice cream. Coconut milk was selected due to its fat content. Date syrup served as a sweetener that is low in sugar content. FOS was included as a fibre that promotes the growth of beneficial bacteria in your intestines. It also substitutes certain sugar. They employed a design to evaluate varying quantities of coconut milk, date syrup, and FOS. They examined how these components influenced the texture, flavour, and colour of the ice creams. They also examined aspects such as its melting properties and antioxidant effectiveness. The optimal combination was determined to be 50% coconut milk, 11.32% date syrup, and 5.81% FOS. This ice cream possessed a flavor and consistency. Here are several specifics: The overrun was 65.14%. The melting rate stood at 40.85%. It contained 66.32% moisture, 18.5% fat, and 2.93% protein. The ice cream contained a variety of components, including total phenols measured at 79.85 mg GAE/100 ml and antioxidant activity recorded at 54.80%. Individuals enjoyed the flavor, consistency, and creaminess of this plant-based ice cream. In certain aspects, it surpassed dairy ice cream. This research indicates that it's possible to create a vegan prebiotic ice cream utilizing coconut milk, date syrup, and FOS. These components offer health advantages. Ensure the ice cream is flavourful. The research demonstrates that FOS acts as a prebiotic in this ice cream.

Kowalczyk et Al. (2022) [80] studied whether adding fibres like inulin and apple fibre to sheep milk ice cream can help protect five different types of good bacteria during digestion. This study was done because sheep milk is nutritious and prebiotic fibres are good at surviving stomach digestion and helping good bacteria grow. They made four types of ice cream using sheep milk: one without any added fibre, one with 4% inulin, one with a mix of 2.5% inulin and 1.5% apple fibre and one with 4% apple fibre. Each type was then added with five different good bacteria. Lacticaseibacillus casei 431, acidophilus LA-5, Lacticaseibacillus paracasei L-26, Lacticaseibacillus rhamnosus and Bifidobacterium animalis ssp. Lactis BB-12. They then tested how well these bacteria survived in a digestive system. The results showed that adding 4% inulin helped Bifidobacterium animalis BB-12 survive better in the stomach with only 16.7% dying off. In the intestine 4% inulin also helped Lacticaseibacillus rhamnosus and Bifidobacterium animalis BB-12 survive by 10.8% and 22% respectively. Adding 4% apple fibre helped

Lacticaseibacillus casei and Lacticaseibacillus paracasei survive by 7.86-11.26%. Most of the bacteria in the ice cream survived well with numbers between 6.11 and 8.88 log cfu/g in the intestine. This is enough to help people feel better. The study found that prebiotic fibres, inulin really helped the good bacteria survive in the ice cream by forming a protective layer during digestion. This means that adding fibres like inulin, FOS and resistant starches to ice cream can help keep the good bacteria alive and healthy, in our gut.

Narala et Al. (2022) [81] investigated using inulin of fat in vegan ice cream made from pea protein. The goal was to decrease saturated levels while keeping the texture good and consumers happy. This is because people are looking for fat plant-based frozen treats more and more.

Vegan ice cream recipes were made using pea protein concentrate as the main protein. Fat was slowly replaced with amounts of inulin. The samples went through processes like mixing, pasteurization, homogenization, aging and freezing. They were checked for hardness , overrun , melting resistance through analysis and melt-down tests and sensory quality using a 9-point hedonic scale. Ice cream with inulin had overrun values. It was creamier, smoother. Melted more slowly than control samples. This was because inulin creates a gel network. This network acts like globules in ice cream. Texture and overall acceptability scores were much higher in samples, with inulin. This shows that inulin is an ingredient. It can replace fat. Serve as a prebiotic fiber. The research clearly showed that inulin can substitute for fat in vegan ice cream. It improves texture. Offers gut health advantages.

Narala et Al. (2022) [82] expanded their study on inulin to see if it could help make vegan yoghurt. They wanted to make a vegan yoghurt ice cream with pea protein that would still have the bacteria in it even after it was frozen. They also wanted it to taste good and have a texture without using any dairy fat. They made vegan yoghurt ice cream with pea protein. Added inulin to it at different amounts. Then they added vegan yoghurt cultures to it. Watched to see how the bacteria grew at different stages of making it. At the time they checked things like how acidic it was, how thick it was and how hard it was. They also asked people what they thought of it like how creamy it was and how it felt in their mouths. The inulin really helped the vegan yoghurt cultures grow. There were more good bacteria in the ice cream that had inulin in it. It also made the ice cream feel nicer and more creamy. People liked the ice cream with inulin in it better so it must have tasted better to them. This study showed that inulin is really good for vegan foods. It helps the good bacteria grow. It makes the texture better. So it makes sense to use

inulin and other ingredients like it such, as FOS and starches from foxnut and jackfruit seed powder in vegan ice cream. These ingredients can help people have gut health and they can make the ice cream taste better and feel nicer in your mouth.

Lumbantobing et Al. (2019) [83] tried to use jackfruit seeds, which are usually thrown away to make vegan ice cream. They make up 90% of the waste from processing jackfruit seeds. The researchers used the seeds as a plant-based milk source. Jackfruit seeds have a lot of stuff like starch, protein, fibre and important minerals. They washed, boiled and peeled the seeds. Then they turned them into milk using a milling process and filtration. The researchers made three types of ice cream. One was a control and the other two used additives to help mix the ingredients. The researchers checked the ice cream for things like fibre, carbs, fat and protein. They also checked how it looked and felt. They even asked 39 people to taste it and give their opinions. They used a test to see if the results were significant. The ice cream made from jackfruit seeds had fibre. It did not have much fat as regular ice cream. One type of ice cream with an additive called lecithin was thicker. This showed that it was better mixed and more stable. The people who tasted the ice cream liked it. The regular ice cream was liked the most.. The jackfruit seed ice cream was still liked a lot. This study showed that jackfruit seeds can be turned into milk. It can be used to make vegan ice cream that's low in fat and high, in fibre. This is good because it uses a part of the jackfruit that would otherwise be wasted.

Aydemir et al. (2025) [84] explored the valorization of fermented hazelnut cake — a protein-dense byproduct from hazelnut oil extraction processed through solid-state fermentation using Aspergillus oryzae — as a functional enrichment component in vegan ice cream, aiming to create a nutritionally improved, sustainable plant-based frozen treat. Three ice cream recipes were created — dairy ice cream (DIC), vegan with unfermented hazelnut cake (HIC), and vegan with fermented hazelnut cake (FHIC) — and thoroughly analyzed for proximate composition, texture, rheological characteristics, melting properties, antioxidant activity (DPPH), volatile compound profile, ACE inhibition activity, and sensory acceptance before and after simulated in vitro digestion. Fermentation greatly enhanced the protein level in FHIC (64.64%) compared to HIC (58.02%) and produced distinctive volatile compounds such as benzaldehyde and 3-methyl-1-butanol; all types displayed non-Newtonian shear-thinning properties. Dairy ice cream had the highest overrun (15.39%) when compared to vegan options (4.01–7.00%), and

FHIC demonstrated significantly elevated post-digestion antioxidant activity (4.73 µmol TE/g) and ACE inhibition (4.85–7.42%) relative to the dairy control, with the caloric values of vegan formulations being 18–30% lower than DIC. The research revealed that fermented plant by-products can create vegan ice cream with enhanced functional characteristics compared to dairy alternatives, although sensory adjustments are still necessary; these results pertain to the current study as they affirm that bases enriched with plant-based protein can offer nutritional and antioxidant profiles that equal or exceed those of traditional dairy ice cream, an expectation also applied to the germinated amaranth milk utilized here.

Fransisca et Al. (2025) [85] sought to create a nutritionally enhanced vegan ice cream utilizing tempeh milk — made from fermented soybeans and providing high-quality plant protein with better digestibility — as the main ingredient, supplemented with moringa leaf flour, renowned for its outstanding levels of vitamins, minerals, essential amino acids, and antioxidant properties. Tempeh was turned into milk by soaking, blending, and filtering, while moringa leaf flour was added in different amounts; ice cream mixtures were created following standard procedures such as pasteurization, aging, and freezing. Afterward, the samples were evaluated for proximate composition (moisture, protein, fat, carbohydrate, ash), antioxidant activity via the DPPH method, and sensory acceptability through a hedonic scale. Moringa leaf flour enrichment notably enhanced protein, iron, calcium, total phenolic content, and DPPH radical scavenging activity in relation to inclusion levels; nevertheless, elevated moringa amounts adversely impacted sensory scores because of a darker green hue and mild bitterness, yet overall acceptability stayed within a tolerable range at moderate levels, while the fermented tempeh base added better protein digestibility and a subtle umami flavor to the mixture. The research validated that high-protein plant-derived milk paired with a beneficial green component can create a nutritionally enhanced vegan ice cream, a concept directly usable in this study where germinated amaranth milk acts as the protein-rich plant-based foundation with the anticipation of offering similar nutritional and functional advancements.

Hidas et al. (2023) [86] investigated the effects of different plant protein types (pea, soy, rice), guar gum, and microbial transglutaminase (MTGase) on the physical and textural properties of vegan ice cream made from hemp milk, driven by the formulation issues of low overrun, quick melting, and subpar texture that often define plant-based frozen desserts in contrast to dairy alternatives. Ice cream formulations based on hemp drink were developed using various plant proteins along with

guar gum and MTGase through conventional ice cream production methods. They were assessed for apparent viscosity, overrun, hardness, melting rate, and rheological characteristics such as elastic modulus (G') and viscous modulus (G''), in addition to sensory evaluations of texture, flavor, and overall acceptability. All samples exhibited shear-thinning non-Newtonian characteristics; guar gum notably enhanced structural stability and raised viscosity, while MTGase facilitated protein cross-linking, leading to a firmer, more cohesive texture; formulations with 25% hemp drink combined with suitable protein and stabiliser levels earned the highest sensory ratings, and increased hemp drink ratios boosted total phenolic content and antioxidant activity, albeit with quicker melting due to the elevated polyunsaturated fatty acid levels in hemp oil. The research emphasized the necessity of suitable stabiliser choice in plant-based ice cream recipes to attain satisfactory physical quality, and directly endorses the incorporation of gond katira (tragacanth gum) in this study as a natural stabiliser anticipated to serve a comparable structural function to guar gum in enhancing texture and melting resistance of the amaranth milk-based vegan ice cream.

Wyrwisz and Pokorski (2025)[87] examined psyllium mucilage (PM)—a natural hydrocolloid abundant in arabinoxylan, sourced from the seed husks of Plantago ovata— as a clean-label, plant-based stabilizer in vegan yogurt ice cream, assessing its effects at three levels (0.25%, 0.5%, and 0.75%) in comparison to reference stabilizers guar gum (GG) and carob gum (CAR) on rheological properties, overrun, melting resistance, color, hardness, and consumer preference. PM was integrated into ice cream mixes made from vegan yogurt and evaluated for characteristics such as apparent viscosity, flow behavior index (n), yield stress, viscoelastic moduli (G', G''), overrun, melting rate, color (L*, a*, b*), and hardness via texture profile analysis, with sensory acceptability assessed by a consumer panel regarding taste, texture, appearance, and overall quality. All formulations demonstrated non-Newtonian shear-thinning characteristics ($n < 1$); PM at 0.75% attained a viscosity of 585.3 mPa·s with the least melting extent (1.20 g/g) and the longest initial dripping duration (67.2 min), suggesting remarkable heat stability; PM 0.5% received the highest consumer approval rating (7.58), statistically similar to GG (7.61), validating its effectiveness as a natural stabilizer, whereas CAR and PM 0.75% lowered scores due to increased hardness or color alteration. This research thoroughly confirmed the effectiveness of natural mucilaginous plant exudates as stabilizers in vegan frozen desserts and directly relates to the current study since gond katira (tragacanth gum) is a chemically akin natural mucilaginous exudate with similar water-binding, gelling, and stabilizing characteristics, and its inclusion is anticipated to yield similar enhancements in texture and melting resistance in the suggested formulation.

Velotto et Al. (2021) [88] sought to decrease the sugar levels and enhance the nutritional quality of traditional dairy and vegan artisanal ice cream by replacing standard sucrose with stevia—a zero-calorie sweetener sourced from the steviol glycosides of Stevia rebaudiana—and adding chia seeds to provide omega-3 fatty acids, dietary fiber, protein, and texture-altering mucilaginous substances. Ice cream recipes were created in two distinct categories — dairy-derived and vegan — where stevia substituted sucrose at suitable levels and chia seeds were added at different rates; the nutritional profile, including sugar, fiber, protein, and omega-3 fatty acid content, was evaluated along with physicochemical aspects (texture, color, melting characteristics) and consumer preferences through sensory evaluation. The use of stevia led to notably reduced total sugar and calorie content without compromising sensory qualities at appropriate stevia concentrations, while the inclusion of chia seeds greatly increased total dietary fiber and omega-3 levels; both dairy and vegan versions maintained textural attributes and consumer acceptability similar to traditional ice cream, with the vegan stevia-chia versions achieving comparable sensory ratings to the dairy ones, validating that functional ingredients can offset the lack of dairy fat. The research showed that natural, zero-calorie sweeteners and functional seeds can enhance health benefits while preserving sensory quality in vegan ice cream, directly backing the choice of monk fruit sweetener (Siraitia grosvenorii) in this study as a natural, calorie-free, high-intensity sweetener instead of refined sugar, anticipating similar health and sensory advantages.

Acar et Al. (2025) [89] created and analyzed vegan ice cream using red kidney bean milk (RKBM), both with and without the heat-tolerant probiotic Bacillus coagulans ATCC 7050, benchmarking it against dairy variants to assess the technological viability and biofunctional advantages of legume-derived vegan frozen treats. Four formulations were created — dairy lacking probiotic (C), dairy containing B. coagulans (CP), vegan RKBM without probiotic (R), and vegan RKBM with B. coagulans (RP) — undergoing fermentation or ripening prior to freezing and hardening; extensive analysis was performed for dry matter, protein, pH, titratable acidity, color (L*, a*, b*), overrun, hardness, antioxidant activity (TEAC), total phenolic content (TPC), and probiotic viability ($\log_{10}$ CFU/g) at various processing stages. Dry matter varied from 33.80 to 35.54%, while protein ranged between 2.05 and 2.20%, showing no significant difference between vegan and dairy samples; vegan samples demonstrated less lightness but greater overrun (36.00–37.50% compared to lower dairy values) and considerably higher antioxidant activity (5224–6148 µM TEAC/g versus dairy) and TPC (72.98–184.75 mg

GAE/100 g), with probiotic viability in RP remaining functionally significant (6.93 $\log_{10}$ CFU/g after freezing), although vegan samples received lower sensory scores overall than dairy samples. The research verified that milk derived from legumes or pseudocereals can produce functional vegan ice cream exhibiting better bioactive and probiotic properties compared to dairy, thereby endorsing the choice of germinated amaranth milk as the protein source in this study, anticipating that it will likewise provide improved antioxidant and functional traits in the final product.

Caponio et Al. (2025) [90] explored the utilization of oat okara — the protein-rich, fiber-filled by-product produced in oat milk manufacturing and often thrown away — as a functional component in plant-based ice cream, aiming to decrease food waste while improving the nutritional, rheological, and sensory attributes of the frozen treat. Oat okara was added to plant-based ice cream recipes at different levels, and samples were analyzed for dietary fiber, protein, fat, and carbohydrate content through proximate analysis, rheological properties such as apparent viscosity and viscoelastic behavior, physicochemical characteristics of melting resistance, overrun, and color, as well as sensory acceptability by a trained panel. The addition of oat okara greatly increased the dietary fibre content across all levels of inclusion, designating the product as a functional food; rheological stability improved at moderate levels of okara, as the water-retaining polysaccharide fraction enhanced viscosity and structural stability, and melting resistance was correspondingly improved; however, elevated okara levels resulted in a somewhat gritty texture that diminished sensory appeal, while moderate inclusion levels retained acceptable ratings. The research confirmed the idea of employing underused plant by-products abundant in dietary fibre as sustainable and functional components in vegan ice cream, directly endorsing the use of jackfruit seed powder in this study — another underutilised by-product that possesses similar fibre-boosting and structural characteristics — to create a nutritionally improved plant-based frozen treat while aiding in waste minimization.

Jan et Al. (2023) [91] carried out an extensive narrative review to compile existing information on the nutritional profile, antinutritional components, health-enhancing properties, and various uses in food, medicine, and cosmetics for amaranth and quinoa, intending to encourage their broader use as functional food ingredients and nutraceuticals in conventional food systems. A thorough investigation of peer-reviewed articles from PubMed, Scopus, and Web of Science was carried out, encompassing research on compositional analysis, the impact of processing methods such as germination, fermentation, and

extrusion on decreasing antinutrients and improving bioavailability, along with recorded health bioactivities. Amaranth grain has been identified to possess 15–16% protein with a comprehensive amino acid profile that is notably better than traditional cereals — particularly elevated in lysine (55–65 mg/g protein) and sulfur-containing amino acids. Its lipid content (6–9%) includes as much as 83% unsaturated fatty acids and remarkably high squalene levels (up to 11% of total lipids), offering cholesterol-lowering and skin-protective benefits. Bioactive phenolic compounds such as quercetin, kaempferol, rutin, ferulic acid, and gallic acid provide antioxidant, anti-inflammatory, antidiabetic (inhibition of DPP-IV and alpha-glucosidase), antihypertensive (ACE inhibition), and hypocholesterolaemic effects. The antinutrients phytate (2.9–7.9 g/kg) and saponins (0.9–4.91 mg/kg) decreased significantly through germination, which simultaneously enhanced protein availability by 8%, total energy by 11%, and reduced resistant starch by 70%, improving both digestibility and bioavailability. The review definitively demonstrated that germinated amaranth is a nutritionally enhanced pseudocereal, offering significantly improved protein bioavailability and lower antinutrient levels. This directly supports the choice of germination pre-treatment for amaranth as the main milk base in this study, scientifically confirming germination as the best processing method to enhance the nutritional quality and functional efficacy of the amaranth milk utilized in this formulation. It seems like you've provided a reference number, but there is no text to paraphrase

Kiełczewska et Al. (2025) [92] sought to assess the physicochemical and sensory compatibility of three high-protein dairy products — micellar casein concentrate (CN), buttermilk protein concentrate (BMP), and whey protein concentrate with 80% protein content (WPC 80) — as functional enhancers in ice cream manufacturing, addressing the increasing consumer interest in high-protein frozen treats and the scarce comparative research regarding the interactions of various protein fractions within the ice cream structure. Ice cream formulations were developed with each protein source substituted at a standard inclusion rate, using skimmed milk powder (SMP) as the reference; the ice cream mixtures were assessed for total protein content, color parameters (L*, a*, b*), and consistency index, whereas the final ice cream samples were examined for overrun, melting rate, hardness, and sensory characteristics by trained evaluators. Among the mixtures, WPC 80 resulted in the darkest shade of ice cream and the lowest overrun values, showing the highest hardness and lowest fluffiness ratings in sensory tests due to the development of a dense three-dimensional β-lactoglobulin network that enhanced viscosity and limited air incorporation during freezing; CN and BMP produced ice creams with enhanced overrun and improved fluffiness ratings, with CN resulting in the lightest color, while

BMP ice cream demonstrated comparable sensory evaluations to the SMP control at a lower cost; protein levels were significantly increased across all fortified formulations compared to the control, with no notable differences in melting rate found between CN and BMP treatments. The research determined that incorporating high-protein dairy preparations into ice cream can enhance its nutritional and functional aspects; however, the selection of protein preparation plays a crucial role in shaping the product's structure, texture, and sensory attributes. These results are closely related to the current research, as germinated amaranth protein—a plant-derived protein with a dominance of albumin and globulin—is expected to engage with the ice cream matrix similarly to these dairy products, making the management of texture and structure through stabiliser incorporation a crucial aspect of formulation.

Cruz and Martinez-Monteagudo (2025) [93] aimed to systematically study how changing the whey protein to casein ratio in ice cream recipes affects rheology, overrun, hardness, meltdown characteristics, and shape retention, intending to create a protein ratio guideline for developing high-protein ice cream with superior physical qualities. Five different whey protein-to-casein ratios — 13:87, 20:80, 33:67, 45:55, and 54:46 — were evaluated within a controlled matrix consisting of 4.5% total protein, 13% total fat, and 42% total solids; each mixture underwent rheological assessments of the ice cream blend, and the resulting ice cream was analyzed for overrun, instrumental hardness using texture profile analysis, meltdown rate, and shape retention through gravimetric methods, all conducted under standardized temperature and conditions. Formulations with increased casein ratios (13:87 and 20:80) demonstrated significantly elevated overrun values of 56.21% and 38.14% respectively, alongside lower hardness measurements of 52.13 N and 58.14 N, whereas ice creams with a higher whey protein composition resulted in a firmer texture at 75.18 N and the minimum overrun of 20.83%; a 1.8-fold delay in meltdown initiation was recorded for the 20:80 whey:casein mix in comparison to the 54:46 formulation, suggesting that casein-rich ice cream effectively delays melting and maintains shape; rheological analysis revealed that mixtures with increased casein proportions displayed higher apparent viscosity and more pronounced shear-thinning characteristics, traits that enhanced air entrapment during freezing and contributed to the overall light texture of the final product. The research showed that casein has a key structural function in ice cream, influencing both the incorporation of air and the melt-resistance of the final product, while increased whey protein levels — despite their nutritional benefits — negatively affect physical quality; these results establish a crucial foundation for recognizing the structural difficulties anticipated in the current study, which involves a plant-based amaranth protein matrix without casein's micellar structure must depend

on gond katira and FOS to make up for the lack of casein-related textural elements.

Bekiroglu et Al. (2024) [94] sought to assess the impact of xanthan gum concentration and oat-based milk substitute (OBMS) level on the rheological properties, overrun, melting characteristics, texture, color, and consumer sensory acceptance of a completely vegan frozen dessert, inspired by the rising popularity of oat milk as a plant-based base and the acknowledged significance of hydrocolloid stabilizers in addressing the textural shortcomings of plant-based frozen desserts in comparison to dairy alternatives. Vegan ice cream recipes were created using oat milk as the main base, with xanthan gum added in different amounts, and evaluated for properties such as apparent viscosity, flow behaviour index, dynamic oscillatory moduli (G', G''), overrun, first drip time, colour metrics (L*, a*, b*), and hardness through texture profile analysis, while sensory panels assessed colour, flavour, texture, creaminess, and general acceptability. All formulations exhibited non-Newtonian shear-thinning flow characteristics typical of viscoelastic frozen dessert matrices; increased concentrations of xanthan gum noticeably raised apparent viscosity and elastic modulus while decreasing overrun and initial drip time, suggesting enhanced structural stability and melting resistance; the natural β-glucan content of oat milk positively influenced viscosity and creaminess versus control formulations without OBMS, and at ideal xanthan gum levels, sensory ratings for creaminess and overall acceptability matched those of traditional ice cream, whereas excessive stabilizer levels resulted in undesirably gummy textures and diminished flavor ratings. The research demonstrated that an oat-derived milk alternative, when paired with a suitable quantity of natural hydrocolloid stabiliser, can create a vegan frozen treat with acceptable physicochemical and sensory properties. These results are particularly pertinent to the current research, where a structurally alike stabiliser — gond katira (tragacanth gum) — is anticipated to serve a similar function in enhancing the texture and melt-resistance of the amaranth milk-based vegan ice cream.

Szydłowska et Al. (2024) [95] performed a narrative review alongside their experimental findings to thoroughly outline the technological, microbiological, and sensory elements influencing the quality of probiotic plant-based frozen desserts. This work addresses the increasing consumer demand for vegan frozen desserts and probiotic functional foods, while also highlighting the inadequate mechanistic knowledge of how plant-based matrices interact with probiotic microorganisms during both processing and storage. The review integrated findings from existing literature with the authors' own studies on plant-based probiotic

frozen desserts, addressing probiotic strain selection, survival dynamics under cold stress and acidity, the protective functions of prebiotic fibers such as inulin, FOS, GOS, and XOS, the influence of cryoprotectants, the effect of plant-based matrix composition (fat levels, protein sources, sugar types) on ice crystal development and probiotic survival, and consumer sensory assessments of various plant-based probiotic frozen desserts. Plant-based matrices were shown to have significant benefits over dairy for probiotic delivery in various areas — particularly their reduced natural bacterial load enabling more controlled fermentation and their polyphenol content offering indirect antioxidant protection to probiotic cells — although they also posed difficulties such as lower buffering capacity and the lack of protective milk proteins that serve as cryoprotectants; the addition of prebiotics, especially inulin and FOS, consistently enhanced probiotic viability by 1–2 log CFU/g during frozen storage while simultaneously improving textural stability by increasing viscosity and gel formation; the required minimum threshold of 10^6 CFU/g for a product to be deemed functional and therapeutic was attainable in plant-based frozen desserts when formulation and processing conditions were optimized. The assessment determined that frozen desserts enriched with plant-based prebiotics constitute a feasible and commercially appealing segment of functional foods that can provide therapeutic doses of probiotics. This supports the reasoning for including prebiotic components like FOS and resistant starches from makhana and jackfruit seed powder in the current formulation, where they are anticipated to act as both structural elements and functional agents to protect and nourish the gut microbiota.

Limjaroen and Kijroongrojana (2024) [96] intended to explore the combined effects of fat content and prebiotic type — galactooligosaccharides (GOS) and fructooligosaccharides (FOS) — on the viability of Bifidobacterium longum TISTR 2195 in yogurt ice cream, as well as the physicochemical, rheological, and microstructural characteristics of the product, focusing on the formulation challenge of maintaining low fat content, satisfactory physical quality, and functionally significant probiotic viability in a frozen synbiotic dessert. Yogurt ice cream was made with two fat levels (2% and 10%) and two types of prebiotics (GOS and FOS) at different concentrations, inoculated with B. longum TISTR 2195. The formulations were assessed for apparent viscosity, flow behavior, dynamic oscillatory rheology, overrun, hardness, melting rate, color, and microstructure of ice crystals and air cells using optical and electron microscopy, along with probiotic viability determined by serial plate count pre- and post-in vitro simulated gastrointestinal digestion. FOS at the optimal level consistently exceeded GOS in preserving probiotic viability throughout 12 weeks of frozen storage, minimizing the log CFU/g reduction during storage and

maintaining viable counts above 10^6 CFU/g at the study conclusion; full-fat formulations containing FOS exhibited the best distribution of ice crystals and air cells, resulting in the highest creaminess ratings; FOS also significantly enhanced apparent viscosity compared to the control, aiding in lower ice recrystallization and better overrun stability; low-fat formulations displayed noticeably poorer textural quality with a coarser microstructure and increased hardness, even though probiotic viability was comparably preserved with FOS included The research indicated that FOS outperforms GOS as a prebiotic in probiotic-infused frozen desserts, providing bifidogenic advantages during gut transit and enhancing the ice cream structure.

Gorman et Al. (2023) [97] aimed to investigate if consumer sensory perception of plant-based frozen desserts varied from traditional dairy ice cream when assessed under blind conditions — without product labels — as opposed to when ingredient lists were available, thereby separating the inherent sensory attributes of plant-based products from the psychological effects of branding, labeling, and ingredient anticipations. Vanilla-flavoured frozen desserts available in the market — including one dairy ice cream, one frozen dairy dessert (FDD), and three plant-based options made from coconut, cashew, and soy bases — were assessed by consumer panels under two scenarios: blind tasting and labelled tasting revealing complete ingredient details; evaluations utilized 9-point hedonic scales for overall liking, flavour, and texture, alongside check-all-that-apply (CATA) sensory profiling to pinpoint descriptors most closely tied to each product. In blind testing, dairy ice cream was rated much more favorably than all plant-based substitutes regarding flavor, texture, and overall appeal; plant-based frozen treats were described with terms like sandy texture, watery mouthfeel, off-flavor, and reduced lubricity—qualities linked to insufficient emulsification of plant proteins, lack of partially coalesced fat structures, and the presence of native plant volatiles that contribute to beany or grassy notes; once ingredient lists were revealed, consumer ratings for plant-based items rose slightly, indicating that positive health and sustainability associations somewhat mitigated sensory dissatisfaction, yet the preference ranking stayed the same; soy-based frozen dessert received the worst ratings under both conditions due to strong beany off-flavor. The research thoroughly illustrated that inherent textural and flavor shortcomings—mainly the gritty mouthfeel and undesirable tastes associated with plant protein matrices—are the main obstacles to consumer acceptance of plant-derived frozen desserts. These results directly influence the choice of ingredients in the current study, where germinated amaranth milk (selected for lower anti-nutritional factors and off-flavours), gond katira (for enhancing texture), and monk fruit

sweetener (to offset lingering cereal bitterness) are anticipated to resolve the sensory deficiencies noted.

Abou Ayana et Al. (2025) [98] sought to create an innovative biofunctional ice cream utilizing enzymatically processed oat milk as a plant-based dairy alternative, confronting the twofold issue of enhancing the physicochemical and nutritional properties of oat milk — which tends to undergo starch gelation and Maillard browning during standard heating methods — while also assessing its ability to preserve Lactobacillus acidophilus viability during frozen storage as a functional probiotic carrier. Oat milk was created using a unique cold-water α-amylase enzymatic method without using heat, effectively hydrolyzing starch to avoid gelation and browning while maintaining β-glucan levels; formulations were designed with oat milk replacing skim milk at 25%, 50%, 75%, and 100% ratios, inoculated with L. acidophilus, and assessed for overrun, viscosity, hardness, melting rate, color, total β-glucan, total phenolic content, antioxidant activity (DPPH), and probiotic viability (log CFU/g) at weeks 0, 4, 8, and 12 during frozen storage. Enzymatic treatment effectively eliminated gelation and browning across all substitution levels, resulting in a smooth, stable oat milk base with uniform viscosity; L. acidophilus viability was well-preserved at 25–50% oat milk substitution, with viable counts consistently exceeding the functional threshold of 10^6 CFU/g over 12 weeks, while 100% oat milk substitution led to reduced probiotic survival due to the lack of dairy proteins that offer cryoprotective buffering; β-glucan content rose proportionally with oat milk concentration, enhancing the functional fiber profile; antioxidant activity and total phenolic content were notably higher in oat milk-based formulations, and sensory panelists reported acceptable taste and texture at 25–75% substitution levels. The research showed that oat milk treated with enzymes can act as a suitable plant-derived foundation for biofunctional ice cream, maintaining probiotic viability while providing clinically significant concentrations of β-glucan, These results are pertinent to the current research, demonstrating that plant-based milk bases treated to reduce antinutritional factors—similar to the germination of amaranth in this study—can promote functional microbiota enhancement and satisfactory physical quality in vegan frozen desserts.

CHAPTER-III
METHODOLOGY

METHODOLOGY

The present study was experimental in design, aimed at formulating and evaluating a prebiotic plant-based high-protein ice cream using germinated amaranth milk, foxnut (makhana), jackfruit seed powder, tragacanth gum (gond katira), and monk fruit sweetener. The study was conducted at NIMS University, Rajasthan, Jaipur over a period of six months. The methodology was carried out in six structured phases covering market survey, procurement and processing of raw materials, product development and standardization, sensory evaluation, proximate and physicochemical analysis, antioxidant potential assessment, shelf-life estimation, and cost and packaging analysis.

PHASE I – Market Survey of Commercially Available Plant-Based and Functional Ice Creams

PHASE II – Procurement and Processing of Raw Materials

PHASE III – Standardization and Development of the Vegan Ice Cream

PHASE IV – Sensory Evaluation of the Developed Product

PHASE V – Proximate, Physicochemical, and Antioxidant Analysis

PHASE VI – Shelf-Life Assessment, Packaging, Labelling, and Cost Analysis

Conduct Market Survey

↓

Procurement of Materials

↓

Processing of Materials

↓

Standardization & Development of Food Product

↓

Sensory Evaluation of Developed Food Product

↓

Analysis of Proximate Parameters, Physicochemical Properties & Antioxidant Potential

↓

Shelf-Life Analysis of Product

↓

Packaging, Labelling & Cost Analysis

Fig 3.1 Flowchart of methodology

PHASE I – Market Survey of Commercially Available Plant-Based and Functional Ice Creams

3.1 Market Survey

Understanding the market is crucial before creating any new food product. To do this a market survey was done to find out what consumers want and need. The survey looked at what ingredients are popular what nutrients are missing in existing products and how aware people are of plant-based and functional ice creams. The survey was done in a way checking out retail stores, supermarkets and online platforms like Amazon and BigBasket. It also looked at brand websites and product information that has been published. The survey collected data on things like product name, brand, ingredients, nutritional claims, price and quantity. This was done for vegan, high-protein and sugar-free ice cream variants that are available in stores in India and other countries.

PHASE II – Procurement and Processing of Raw Materials

3.2 Study Area

The study was conducted in the cookery laboratory of NIMS University, Jaipur, Rajasthan. All product development, standardization, and sensory evaluation activities were carried out at the same location. Laboratory analyses for proximate composition, physicochemical properties, and

antioxidant activity were performed in an accredited laboratory using standard analytical instruments.

3.3 Procurement of Raw Materials

All raw materials used in this study were procured from reliable local markets in Jaipur and verified online retail platforms (Amazon India, Confect). The following ingredients were sourced: amaranth grains (for germinated amaranth milk), foxnut (makhana), jackfruit seeds (for jackfruit seed powder), tragacanth gum (gond katira), monk fruit sweetener, almond butter, cocoa powder, walnuts, almonds, cashews, and dark chocolate (70% cocoa preferred). Each batch of raw material was screened prior to use for freshness, cleanliness, and freedom from visible spoilage or infestation. Standardised lot sizes were maintained throughout the study to ensure batch-to-batch consistency.

3.4 Processing of Raw Materials

Each ingredient was pre-processed individually before being incorporated into the ice cream formulation. The processing methods are described below.

3.4.1 Processing of Amaranth Seeds for Germinated Amaranth Milk

Germination was carried out to improve protein bioavailability, reduce antinutritional factors, and enhance the overall nutritional quality of amaranth milk. The process involved the following steps:

- Amaranth grains were manually sorted and cleaned to remove foreign matter, damaged seeds, and extraneous material.
- The cleaned grains were rinsed three to four times with clean water and then soaked in distilled water for 8–12 hours at room temperature.
- After soaking, excess water was drained completely and the grains were spread evenly on moist muslin cloth or filter paper placed in germination trays.

- The trays were covered with another layer of moist muslin cloth to maintain humidity and incubated at 28–30°C in dark conditions for 24–48 hours to allow germination.
- After germination, the sprouts were gently washed with clean water to remove any surface microorganisms.
- The germinated sprouts were ground with distilled water using a high-speed blender and the resulting slurry was filtered through a clean double-layered muslin cloth to obtain amaranth milk.
- The milk was pasteurised at 72°C for 15–20 seconds using a water bath and immediately cooled to 4°C for storage until further use.

Cleaning of amaranth grains
↓
Soaking (8–12 hours)
↓
Spreading on moist filter paper/muslin
↓
Covering and incubation (28–30°C, 24–48 hrs)
↓
Germination of grains
↓
Grinding with distilled water
↓
Filtering to obtain amaranth milk
↓
Pasteurization (72°C, 15–20 sec)
↓
Cooling and storage at 4°C

Figure 3.2 – Flowchart of Germinated Amaranth Milk Processing
[99]

3.4.2 Processing of Foxnut (Makhana)

- Foxnuts were cleaned to remove any dust, debris, or discoloured pieces.

- The cleaned foxnuts were lightly roasted in a dry iron pan over medium heat until they expanded and became crunchy.
- After cooling to room temperature, they were either incorporated whole as inclusions or lightly ground into a coarse powder, depending on the formulation variation, to enhance protein and starch contribution.

3.4.3 Processing of Jackfruit Seeds for Jackfruit Seed Powder

- Fresh jackfruit seeds were collected, washed thoroughly under running water, and boiled for 15–20 minutes to soften and loosen the outer seed coat.
- The white seed coat was removed after boiling and the inner seeds were sliced into thin pieces.
- The sliced seeds were dried in a hot air oven at 50–60°C until a constant weight was achieved.
- The dried pieces were ground into fine powder using a laboratory grinder and sieved through an 80-mesh sieve.
- The powder was packed in airtight containers and stored at room temperature until use.

3.4.4 Processing of Gond Katira (Tragacanth Gum)

- Good-quality gond katira flakes were selected and inspected for freedom from discolouration or contamination.
- The flakes were soaked in distilled water for 12–24 hours at room temperature to allow complete hydration and gel formation.
- The gel was stirred periodically to break up any lumps and ensure a smooth, uniform consistency.
- If undissolved particles were observed, the gel was filtered through a fine sieve before incorporation.
- The hydrated gum gel was added to the ice cream mix during the stabiliser incorporation stage, before pasteurisation.

3.4.5 Processing of Almonds and Cashews

- Almonds and cashews were soaked overnight in water to soften and reduce antinutritional factors.
- Almonds were peeled after soaking to remove the brown skin.
- Both nuts were then blended with a small quantity of water to form smooth nut pastes or nut milks for incorporation into the mix to enhance creaminess and protein content.

3.4.6 Processing of Dark Chocolate

- Dark chocolate (70% cocoa) was chopped and melted at 40–45°C using a double boiler to a smooth, lump-free consistency.
- The melted chocolate was tempered if required to improve texture and flavour, and added to the ice cream mix during the blending stage.

3.4.7 Processing of Walnuts

- Walnuts were lightly roasted at 100–120°C for 5–7 minutes to enhance flavour and reduce oxidative rancidity.
- After cooling, they were coarsely chopped or ground before addition to the ice cream mix.

3.4.8 Processing of Cocoa Powder

- Food-grade cocoa powder was inspected, sieved through a fine sieve to ensure uniform particle size, and dry-roasted lightly at low temperature if required to enhance flavour.
- The processed cocoa powder was stored in an airtight container until use.

3.4.9 Processing of Almond Butter

- Raw almonds were cleaned, soaked for 6–8 hours, and peeled.

- The peeled almonds were lightly roasted, cooled, and then ground in a food processor until a smooth, creamy butter was obtained.
- The almond butter was stored under refrigeration until incorporation into the ice cream mix.

3.4.10 Processing of Monk Fruit Sweetener

- Commercially available, food-grade monk fruit sweetener was selected and checked for purity and absence of moisture clumping.
- The sweetener was dissolved in a small quantity of warm water to ensure uniform distribution in the ice cream mix.
- The prepared solution was filtered if required and stored in a clean, airtight container until use.

PHASE III – Standardization and Development of the Vegan Ice Cream

3.5 Standardization of the Product

Standardization refers to the systematic process of identifying the most suitable combination of ingredients and processing conditions to achieve a consistently acceptable product in terms of taste, texture, colour, and nutritional quality. In this study, standardization was carried out through a pilot study and the development of multiple formulation variations, which were subsequently evaluated by sensory panels.

3.5.1 Pilot Study

An initial pilot study was conducted using a hit-and-trial approach with small-scale batches (approximately 200 g per batch) to establish the feasibility of the formulation and assess the overall taste, texture, colour, and aroma of the base ice cream. Observations from the pilot study were used to refine ingredient proportions and processing conditions before developing the final set of variations.

3.5.2 Development of Formulation Variations

Four formulations of the vegan ice cream were prepared — one control and three variations (AAA, BBB, CCC) — by altering the proportions of the two key functional ingredients i.e. (cashews and gond katira) while keeping the base ingredients (germinated amaranth milk, monk fruit sweetener, etc) constant. This approach allowed a systematic comparison of the effect of varying ingredient ratios on sensory quality and nutritional composition. The control formulation was prepared using standard proportions as identified in the pilot study, while AAA, BBB, and CCC incorporated progressively different levels of makhana, jackfruit seed powder, and nut pastes to identify the most acceptable combination. The formulation chart is presented in Table 3.1.

Table 3.1: Formulation Chart for Vegan Ice Cream Variations (per 500-515 g base)

Ingredient	AAA	BBB	CCC
Germinated Amaranth Milk (ml)	250ml	250ml	250ml
Makhana powder (g)	10g	10g	10g
Jackfruit seed powder (g)	8g	8g	8g
Gond katira (hydrated gel, g)	35g	45g	55g
Monk fruit sweetener (g)	7g	7g	7g
Almond butter (g)	25g	25g	25g
Thick coconut milk (ml)	45ml	45ml	45ml
Dark chocolate (g)	25g	25g	25g
Cocoa powder (g)	10g	10g	10g
Cashews (g)	70g	85g	55g

Vanilla extract	3.75 ml	3.75 ml	3.75 ml

3.5.3 Ice Cream Preparation Method

The ice cream was prepared following standard manufacturing steps with modifications suitable for a plant-based, stabiliser-enriched formulation. The process is described in the steps below and illustrated in Figure 3.3.

- All raw materials were weighed accurately using a calibrated weighing balance.
- The germinated amaranth milk was taken as the base liquid. Nut pastes (almond, cashew) were blended with the amaranth milk to form a smooth, homogeneous base mix.
- Makhana powder and jackfruit seed powder were added to the base mix and stirred well to avoid lumping.
- The hydrated gond katira gel was incorporated as the stabiliser and blended thoroughly into the mix.
- Cocoa powder or melted dark chocolate was added for flavour.
- Monk fruit sweetener solution was added to the mix and mixed until uniformly distributed.
- The complete ice cream mix was pasteurised at 72°C for 15–20 seconds in a water bath, then cooled immediately to 4°C.
- The cooled mix was homogenised by blending at medium speed to ensure uniform fat distribution and air incorporation.
- The homogenised mix was aged at 4°C for 6–12 hours to allow the stabiliser to fully hydrate and develop proper viscosity.
- The aged mix was frozen in a domestic ice cream maker (churning) at −18°C to −20°C until a soft-serve consistency was achieved, incorporating approximately 30–40% overrun.
- The semi-frozen product was transferred to labelled airtight food-grade containers and hardened at −18°C to −20°C for 6–8 hours before evaluation.

Selection and cleaning of raw ingredients

↓

Processing of individual ingredients (as above)

↓

Preparation of amaranth milk (base)

↓

Incorporation of nut pastes (almonds, cashews, walnuts)

↓

Addition of makhana powder and jackfruit seed powder

↓

Hydration of tragacanth gum (stabilizer) and incorporation

↓

Melting and mixing of dark chocolate

↓

Blending all components into smooth mixture

↓

Pasteurization at 72°C for 15–20 seconds

↓

Cooling to 4°C

↓

Homogenization for uniform texture

↓

Aging of mix at 4°C for 6–12 hours

↓

Freezing with incorporation of air (overrun)

↓

Hardening at -18°C to -20°C

↓

Storage at -18°C until consumption

Figure 3.3 – Flowchart of ice cream preparation process

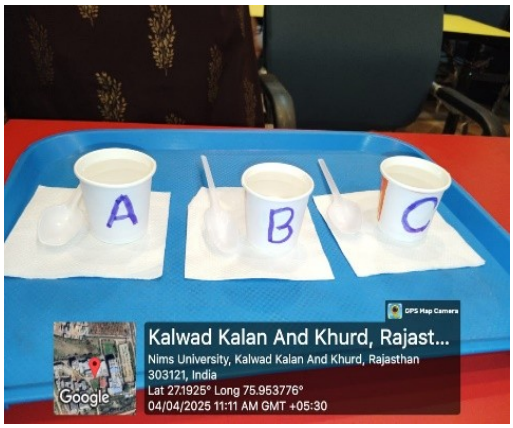
PHASE IV – Sensory Evaluation of the Developed Product

3.6 Sensory Evaluation

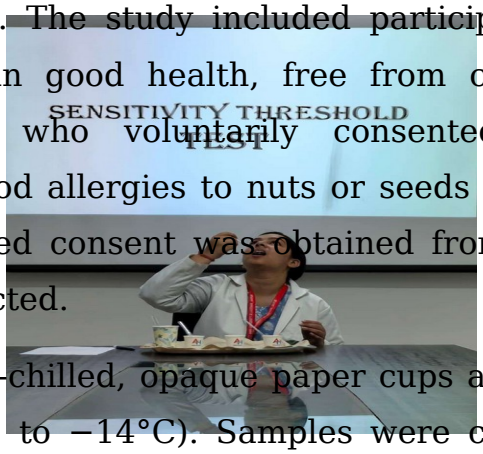
Sensory evaluation is an important scientific tool used to measure how human senses of taste, smell, appearance, and texture perceive a food product. It plays a central role in assessing consumer acceptability and ensuring that the developed ice cream not only meets nutritional targets but is also enjoyable to consume. Two sequential sensory tests were conducted in this study.

3.6.1 Sensitivity Threshold Test

Before proceeding with formal sensory evaluation, a sensitivity threshold test was administered to identify suitable panelists with the ability to detect subtle differences in taste, texture, and flavour intensity. The test was conducted among 30 volunteers from NIMS University who had no known taste-related disorders or conditions. Sugar solutions of varying concentrations were prepared and presented to participants in random order. Participants were instructed to rinse their mouth with plain water before tasting each sample. Each participant recorded the level of sweetness detected for each sample using the sensitivity threshold proforma (Annexure 1). Results were statistically analysed using mean and standard deviation. Panelists who demonstrated adequate sensitivity were selected for the nine-point hedonic test.



(i)



(ii)

Figure 3.4 – (i) Sugar solution with varying concentrations (ii) Panelists performing the sensitivity threshold test

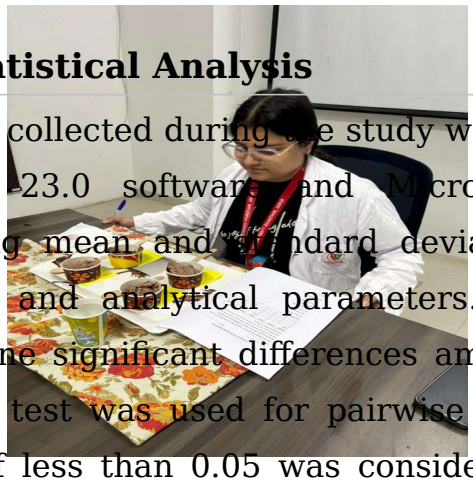
3.6.2 Nine-Point Hedonic Rating Scale

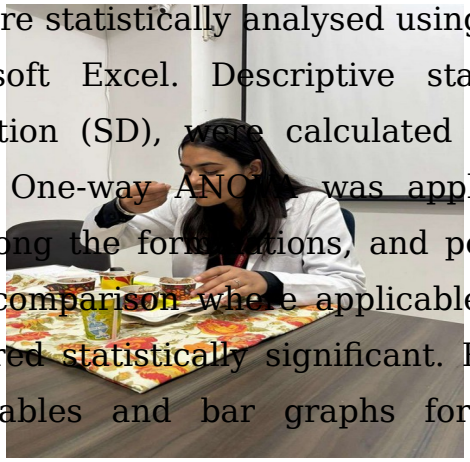
The four formulations — one control (C) and three variations (T1, T2, T3) — were evaluated by a panel of 15 semi-trained individuals selected through the sensitivity threshold test. The study included participants who were above 18 years of age, in good health, free from colds, allergies, or taste disorders, and who voluntarily consented to participate. Individuals with known food allergies to nuts or seeds were excluded from the evaluation. Informed consent was obtained from all participants before the test was conducted.

Ice cream samples were served in pre-chilled, opaque paper cups at the standard serving temperature (−12°C to −14°C). Samples were coded with single-letter codes (C, T1, T2, T3) and presented to panelists in randomised order on a tray along with a glass of plain water at room temperature for palate cleansing between samples. Panelists were seated in a well-ventilated room with adequate lighting and evaluated each sample independently.

Figure 3.5 - (i), (ii) Selected panelists performing the sensory evaluation.

(iii) Developed product with control





3.7 Statistical Analysis

All data collected during the study were statistically analysed using SPSS version 23.0 software and Microsoft Excel. Descriptive statistics, including mean and standard deviation (SD), were calculated for all sensory and analytical parameters. One-way ANOVA was applied to determine significant differences among the formulations, and post-hoc Tukey's test was used for pairwise comparison where applicable. A p-value of less than 0.05 was considered statistically significant. Results were presented in the form of tables and bar graphs for clear interpretation.

3.7.1 Mean

The mean was calculated as the arithmetic average of all observations for each parameter. It was used to represent the central tendency of sensory scores and analytical data across panelists and replicates. All calculations were performed using Microsoft Excel.

3.7.2 Standard Deviation

Standard deviation (SD) was calculated to quantify the variability or dispersion of the data around the mean. A lower SD indicated greater consistency and reproducibility in the results. SD was computed using the standard formula in Microsoft Excel and SPSS.

PHASE V – Proximate, Physicochemical, and Antioxidant Analysis

3.8 Proximate Analysis of the Developed Product

The most acceptably rated formulation, as determined by sensory evaluation, was selected for detailed nutritional analysis. Proximate composition was determined to assess the basic nutritional profile of the developed ice cream. All analyses were performed in triplicate, and results are expressed as mean ± standard deviation. The methods used are listed in Table 3.3.

Table 3.3: Proximate Analysis Parameters and Methods

Parameter	Method	Reference
Moisture content	Hot air oven method	AOAC (2023)
Total protein	Kjeldahl method	AOAC (2023)
Total fat	Soxhlet extraction method	AOAC (2023)
Total carbohydrate	By difference (100 − protein − fat − ash − moisture)	AOAC (2023)
Crude fibre	McCleary acid–alkali digestion method	AOAC (2023)

Total ash	Muffle furnace incineration at 550°C	AOAC (2023)
Total solids	Gravimetric method	AOAC (2023)
Total energy	Atwater conversion factors	AOAC (2023)

3.8.1 Determination of Moisture Content

Moisture content was determined by the hot air oven drying method as per AOAC (2023). Porcelain crucibles were dried at 100°C for 30 minutes, cooled in a desiccator, and weighed as W1. A known quantity of sample was placed in each crucible and weighed (W2). The crucible with sample was then dried at 100°C for 4–6 hours, cooled in the desiccator, and reweighed repeatedly until a constant weight (W3) was obtained. Moisture content was calculated using the formula:

$$\textbf{\textit{Moisture \% = [(W2 − W3) / (W2 − W1)] × 100}}$$

Where W1 = weight of empty crucible, W2 = weight of crucible + sample before drying, W3 = weight of crucible + sample after drying.

3.8.2 Determination of Total Protein

Protein content was estimated by the Micro-Kjeldahl method (AOAC, 2023). The method involves three steps: digestion, distillation, and titration.

Digestion: A 0.5 g sample was weighed into the Kjeldahl digestion tube and a catalyst mixture of potassium sulphate and cupric sulphate (5:1) was added. The sample was heated on the digestion apparatus until the solution turned clear and colourless, indicating complete oxidation of organic nitrogen.

Distillation: The digested sample was transferred to a steam distillation apparatus and 20 ml of 40% sodium hydroxide solution was added.

Ammonia liberated during distillation was trapped in a 100 ml conical flask containing 4% boric acid with 2–3 drops of methyl red indicator.

Titration: The ammonia absorbed in boric acid was titrated against 0.1 N hydrochloric acid. The endpoint was indicated by a change in colour from green to pink. Nitrogen percentage and crude protein were calculated using:

$$\textbf{\textit{Nitrogen \% = [14 × N (HCl) × V (sample − blank) × 100] / Weight of sample (g)}}$$

$$\textbf{\textit{Protein \% = Nitrogen \% × 6.25}}$$

3.8.3 Determination of Total Fat

Fat content was determined by Soxhlet extraction (AOAC, 2023). Pre-weighed thimbles containing 1 g of dried sample were placed in the Soxhlet apparatus with petroleum ether (BP 60–80°C) in pre-weighed aluminium canisters. Extraction was carried out for 2–3 hours at a rate of 2–3 drops per second. The canisters were then dried at 100°C to remove residual solvent, cooled in a desiccator, and weighed to constant weight. Fat content was calculated as:

$$\textbf{\textit{Fat \% = [(W3 − W2) / W1] × 100}}$$

Where W1 = weight of sample, W2 = weight of empty canister, W3 = weight of canister with extracted fat.

3.8.4 Determination of Total Ash

Ash content was determined by muffle furnace incineration (AOAC, 2023). A 2 g sample was placed in a pre-weighed porcelain crucible and incinerated at 550°C for 6 hours until white or greyish ash remained. The crucible was cooled in a desiccator, weighed, and ash percentage calculated as:

$$\textbf{\textit{Ash \% = (Weight of ash / Weight of sample) × 100}}$$

3.8.5 Determination of Crude Fibre

Crude fibre was estimated by the acid–alkali wash method (McCleary method, AOAC 2023). A 2.5 g sample was subjected to sequential digestion with boiling sulphuric acid (200 ml) for 30 minutes followed by washing with hot water and then boiling with sodium hydroxide solution (200 ml) for 30 minutes. The residue was filtered through a Gooch crucible, washed with warm water and 10 ml alcohol, dried at 105°C to constant weight, and then incinerated in a muffle furnace at 600°C for 30 minutes. Crude fibre was calculated as the difference between the weight of dry residue (W1) and the weight of ash after incineration (W2):

$$\textbf{\textit{Crude Fibre \% = [(W1 − W2) / Weight of sample] × 100}}$$

3.8.6 Determination of Total Carbohydrate

Total carbohydrate was calculated by the difference method (AOAC, 2023) using the formula:

$$\textbf{\textit{Carbohydrate \% = 100 − (Moisture \% + Protein \% + Fat \% + Ash \% + Crude Fibre \%)}}$$

3.8.7 Estimation of Total Energy

The energy value of the developed ice cream was calculated from the macronutrient composition using Atwater's conversion factors:

$$\textbf{\textit{Energy (kcal) = [Protein (g) × 4] + [Carbohydrate (g) × 4] + [Fat (g) × 9]}}$$

3.9 Physicochemical Properties of the Product

Physicochemical properties describe the physical and chemical quality characteristics of the ice cream, which directly influence its sensory acceptability and storage stability. The parameters assessed and the methods used are listed in Table 3.4.

Table 3.4: Physicochemical Analysis Parameters and Methods

Parameter	Method	Reference

pH	Digital pH meter	AOAC (2023)
Total Soluble Solids (TSS)	Hand/digital refractometer (°Brix)	AOAC (2023)
Overrun (%)	Weight–volume method	AOAC (2023)
Melting rate	Gravimetric melt-down method	AOAC (2023)
Colour analysis	Colorimeter / Hunter Lab (L*, a*, b*)	AOAC (2023)

3.9.1 pH

The pH of the most accepted ice cream formulation was measured using a calibrated digital pH meter (Elico LI-127). The probe was rinsed with distilled water between readings. Measurements were taken in triplicate on the sample at room temperature and mean values were recorded.

3.9.2 Total Soluble Solids (TSS)

Total soluble solids were measured using a hand or digital refractometer and expressed in degrees Brix (°Brix). A small quantity of the melted ice cream sample was placed on the prism of the refractometer, and the reading was noted in triplicate.

3.9.3 Overrun

Overrun is the increase in volume of ice cream over the volume of the mix used, expressed as a percentage. It reflects the amount of air incorporated during the freezing and churning process. Overrun was calculated using the weight–volume method:

$$\textbf{\textit{Overrun \% = [(Weight of mix − Weight of ice cream) / Weight of ice cream] × 100}}$$

3.9.4 Melting Rate

Melting behaviour was assessed by the gravimetric melt-down method. A weighed quantity of ice cream was placed on a wire mesh positioned over

a beaker at room temperature (25°C). The weight of the melted drip collected in the beaker was recorded at regular intervals (every 10 minutes for 60 minutes) and melting rate was expressed as grams of melt per minute.

3.9.5 Colour Analysis

Colour was measured using a colorimeter with the Hunter Lab colour scale, reporting L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) values. The instrument was calibrated with standard white and black tiles before readings. Measurements were taken in triplicate.

3.10 Antioxidant Potential of the Developed Product

Antioxidant potential was evaluated to quantify the ability of the product to scavenge free radicals and assess its health-promoting value. The parameters and methods used are presented in Table 3.5.

Table 3.5: Antioxidant Analysis Parameters and Methods

Parameter	Method	Reference
Total Phenolic Content (TPC)	Folin–Ciocalteu method, UV-Vis spectrophotometer	AOAC (2023)
DPPH Radical Scavenging Activity	DPPH assay at 517 nm, UV-Vis spectrophotometer	AOAC (2023)

3.10.1 Total Phenolic Content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu colorimetric method. A known quantity of the ice cream extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution. After

incubation at room temperature for 30 minutes, absorbance was measured at 765 nm using a UV-Vis spectrophotometer. TPC was expressed as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g) using a gallic acid standard curve.

3.10.2 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured by the method of Brand-Williams et al. A sample extract was added to a methanolic solution of DPPH (0.1 mM). After 30 minutes of incubation in the dark at room temperature, absorbance was measured at 517 nm. Results were expressed as percentage inhibition using the formula:

% Inhibition = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100

PHASE VI – Shelf-Life Assessment, Packaging, Labelling, and Cost Analysis

3.11 Shelf-Life Assessment

The shelf life of the developed ice cream formulation was assessed during frozen storage at −18°C. The evaluation was conducted at fixed time intervals: Day 0 (initial), Day 15, Day 30, and Day 60. At each interval, samples were assessed for microbial safety and selected physicochemical parameters to detect any changes in product quality over time. The analyses performed are described below.

3.11.1 Total Plate Count (TPC) Method

The Total Plate Count method was used to assess the total viable microbial load in the ice cream at each storage interval. A measured quantity of ice cream was diluted serially using sterile saline. An aliquot of each dilution was spread uniformly onto nutrient agar plates using a sterile spreader. Plates were incubated at 30–37°C for 24–48 hours. After

incubation, colonies were counted manually and results expressed as colony-forming units per gram (CFU/g). Samples were considered safe if counts remained within regulatory limits throughout the storage period. [3]

3.11.2 Yeast and Mould Count

Yeast and mould counts were assessed using selective Sabouraud dextrose agar medium. Sample dilutions were spread on the agar and plates were incubated at 25–30°C for 3–5 days. Colonies were counted and results expressed as CFU/g. Counts were compared against permissible limits stipulated by the Food Safety and Standards Authority of India (FSSAI) to confirm product safety throughout the storage period. [3]

3.11.3 pH and Melting Rate during Storage

To track physicochemical stability, pH and melting rate were also monitored at each storage interval as described in Sections 3.9.1 and 3.9.4 respectively. Any significant change in pH or melting rate over the storage period was noted and reported.

3.12 Nutritive Value Analysis using Indian Food Composition Table

The nutritive value of the individual ingredients used in the most accepted formulation was calculated using the Indian Food Composition Table (IFCT), 2017. The quantity of each ingredient per serving was multiplied by the nutrient composition factor from IFCT to determine the contribution of each ingredient to the total macronutrient and energy content of the product. The calculated values were compared against Recommended Dietary Allowances (RDA) for adults as per ICMR guidelines.

3.13 Cost Analysis of the Developed Product

A cost analysis was performed to determine the economic viability and commercial feasibility of the formulated ice cream. The total cost of production per serving (500 ml) was calculated by accounting for the current market price of each ingredient, packaging material, overhead costs, and profit margin. Overhead charges (labour, fuel, equipment

depreciation, and taxes) were fixed at 20% of total ingredient cost, and a profit margin of 20% was also applied. The formula used for cost estimation was:

Cost of production = Cost of Ingredients + Packaging Cost (Rs) + Overhead (Rs) + Profit (Rs) + Amount of Product (Rs)

The breakdown of production cost is presented in Table 3.6.

Table 3.6: Cost Components of the Developed Vegan Ice Cream (per 500 g serving)

Cost Component	Details	Calculation	Estimated Cost (₹)
Raw Material	Germinated amaranth seeds, Makhana (foxnut), Jackfruit seed powder, Gond katira (tragacanth gum), Monk fruit sweetener, cocoa powder , dark chocolate , cashews , vanilla essence	Quantity of each ingredient used per kg of product × Market price per kg (₹/kg)	₹ ___
Processing	Labour, Utilities (electricity & water), Equipment depreciation (ice cream maker, homogeniser, blast freezer)	All processing-related expenses per kg of product produced	₹ ___
Packaging	Food-grade tubs/cups, Lids/seals, Nutritional labels (FSSAI compliant), Outer cartons	Cost per unit × Number of units produced per kg of product	₹ ___
Regulatory	Physicochemical & microbiological testing, FSSAI certification, Nutritional labelling	Compliance expenses per batch or per year,	₹ ___

Cost Component	Details	Calculation	Estimated Cost (₹)
	compliance	apportioned per kg produced	
Unit Cost	**Total production cost per unit of vegan prebiotic ice cream (per 100 g serving)**	**Total cost (A+B+C+D) ÷ Number of units produced**	**₹ ___**

3.14 Packaging and Labelling

The developed ice cream was packed in airtight, food-grade containers of appropriate size and suitable for frozen storage at −18°C. Packaging materials were selected to prevent moisture ingress, freezer burn, and contamination throughout the product's intended shelf life. A nutritional label was designed using Canva design software in compliance with the Food Safety and Standards (Labelling and Display) Regulations, 2020, as prescribed by the Food Safety and Standards Authority of India (FSSAI). The label included the following mandatory information:

- Product name and description
- Net weight and serving size
- List of ingredients in descending order of proportion
- Nutritional information per 100 g and per serving
- Declaration of allergens (nuts, seeds)
- Vegan and plant-based declaration
- Date of manufacture and best before date
- Storage instructions (Keep frozen at −18°C)
- FSSAI licence number
- Name and address of the manufacturer

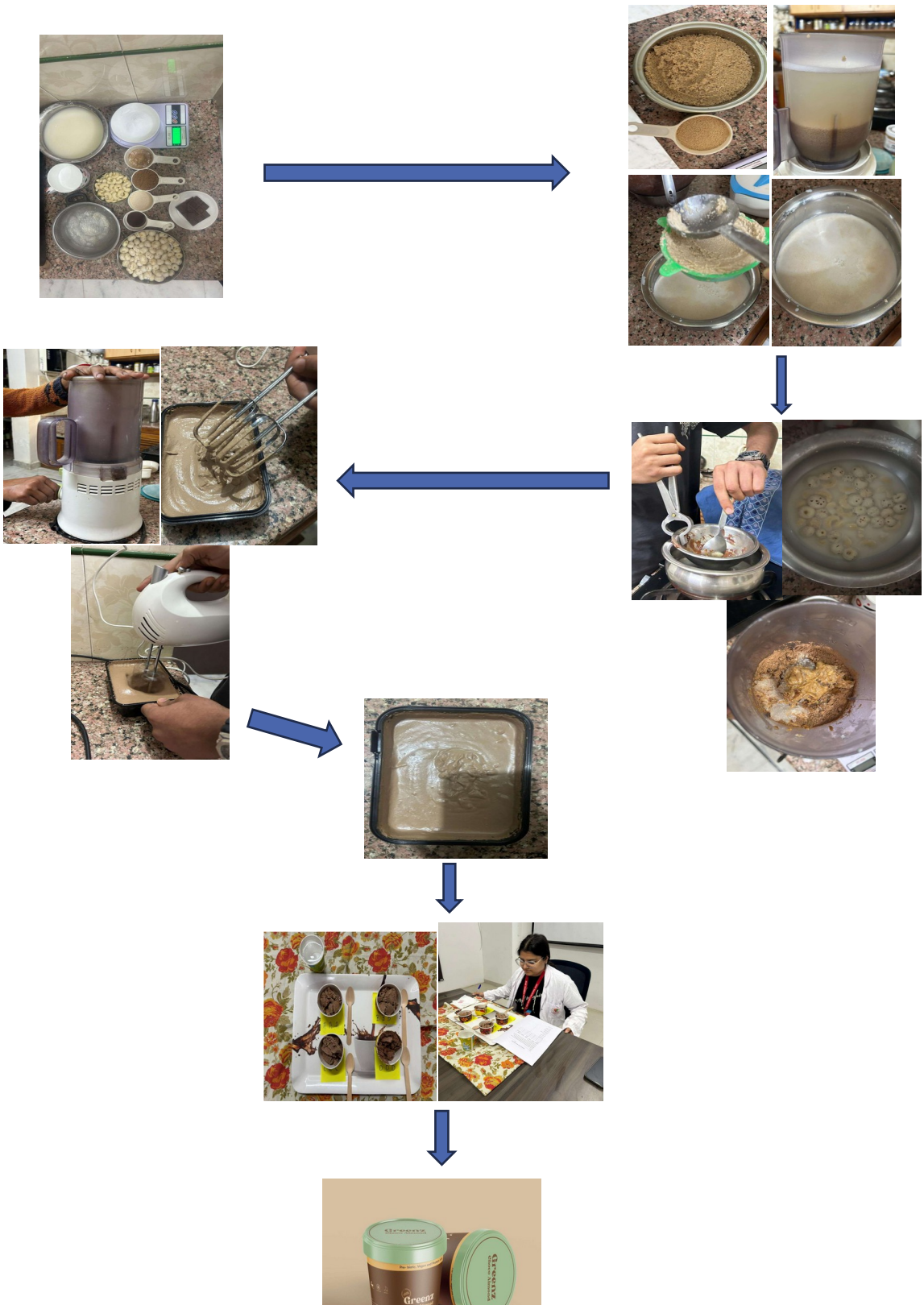


Figure 3.6 Outline of research design

CHAPTER - IV

RESULTS AND DISCUSSION

PHASE 1 – MARKET SURVEY OF COMMERCIALLY AVAILABLE VEGAN AND FUNCTIONAL ICE CREAM PRODUCTS

4.1 Market Survey of Commercially Available Products

The survey showed that while many brands offer vegan or reduced-sugar options, most use almond, coconut, or oat milk as their plant-based base. No product was found that specifically uses germinated amaranth milk as a protein source. The mix of amaranth milk, makhana, jackfruit seed powder, gond katira, and monk fruit sweetener in one formulation is designed to be high in protein, prebiotic, and friendly for those with allergies. This creates a clear gap in the current market. This finding supports the development of the proposed product. The results of the survey were put together and are shown in Table 4.1, below.

Table 4.1: Market Survey of Commercially Available Plant-Based and Functional Ice Cream Products

Brand	Key Ingredients	Claim	Price (₹)	Quantity
Get A Way	Milk, whey protein isolate, cocoa solids, cream, erythritol	High protein, no added sugar	₹135	100 ml
Go Zero	Milk solids, maltitol, stevia, fructooligosaccharide, emulsifiers, cocoa	Zero sugar, low calorie	₹60	100 ml
NOTO Vegan Ice Cream	Water, hazelnut butter, erythritol, maltitol, FOS, coconut milk, coconut oil, cocoa, stevia	Vegan, high protein, zero sugar	₹440	500 ml

Papa Cream 	Almond milk, coconut cream, dark chocolate, cocoa powder, stabilizers	Vegan, sugar free	₹135	100 ml
Brooklyn Creamery 	Water, maltitol, stevia, FOS, cocoa solids, stabilizer (440)	Vegan, no added sugar	₹399	450 ml
Minus 30 	Almond milk, almond butter, chocolate, cocoa butter, stevia	Vegan, sugar free	₹299	115 ml
Nomou 	Coconut milk, coconut cream, palm sugar, cocoa powder	Vegan	₹625	500 ml

Ben & Jerry's (Oat Milk) 	Oat milk, corn syrup, coconut oil, cocoa, fava bean protein, pea protein, guar gum	Vegan, plant-based	₹1742	420 g
Halo Top 	Skim milk, eggs, erythritol, prebiotic fiber, milk protein, organic stevia, guar gum	Protein rich, prebiotic	~₹600	473 ml
Mercely’s prebiotic ice cream 	milk, sugar, milk fat. skim milk powder, fructooligosacceride , emulsifiers, stabilizers, probiotic cultures, cocoa , caramel , natural flavouring agents	Prebiotic	~₹185	500ml
Koozies 	vegan (Orange Fruit and Pulp, Fructooligosaccharide (Prebiotic fibers), Fruit Juice, Salt, Permitted Emulsifying and	vegan and high protein	~₹79	1 stick

	Stabilizing Agents) Protein rich (Milk, Fructooligosaccharide (Prebiotic fibers), Milk Solids, Cocoa Powder, Dark Compound, Whey Protein Concentrate, Choco Syrup, Permitted Emulsifying and Stabilizing Agents, Added Flavor (Chocolate)			

PHASE 2 – IDENTIFICATION, PROCUREMENT, AND PROCESSING OF INGREDIENTS

4.2 Identification and Procurement of Ingredients

The current study examined the creation of a vegan, high-protein ice cream made from germinated amaranth (Amaranthus hypochondriacus) milk as the main base. It also included makhana (foxnut; Euryale ferox), jackfruit seed powder (Artocarpus heterophyllus), gond katira (tragacanth gum; Astragalus spp.), monk fruit sweetener (Siraitia grosvenorii) , cashews, almond butter, thick coconut milk, dark chocolate, cocoa powder, and vanilla extract. We sourced all raw materials from local stores and online platforms in Jaipur, Rajasthan. Amaranth seeds were germinated before extracting the milk to lower antinutrient levels, improve protein absorption, and boost digestibility, according to previous studies (Jan et Al., 2023). The choice of each ingredient was based on their known nutritional, functional, and bioactive properties.

Figure 4.1 — Ingredients Used in the Vegan Prebiotic High-Protein Ice Cream

PHASE 3 – STANDARDISATION AND FORMULATION OF VEGAN PREBIOTIC ICE CREAM

4.3 Standardisation and Formulation Variations

The standardization process involved developing three ice cream formulas: and three variations were (AAA, BBB, CCC). In these formulas, the amount of two key functional ingredients, cashews and gond katira (hydrated gel), was changed while the base ingredients remained the same. These base ingredients included germinated amaranth milk, makhana powder, jackfruit seed powder, monk fruit sweetener, almond butter, thick coconut milk, dark chocolate, cocoa powder, and vanilla extract. The formulation chart for all variations is shown in Table 4.2.

Table 4.2: Formulation Chart for Vegan Prebiotic Ice Cream Variations (per 500–515 g base)

Ingredient	AAA	BBB	CCC
Germinated Amaranth Milk (ml)	250 ml	250 ml	250 ml
Makhana Powder	10 g	10 g	10 g

(g)			
Jackfruit Seed Powder (g)	**8 g**	**8 g**	**8 g**
Gond Katira – hydrated gel (g)	**35 g**	**45 g**	**55 g**
Monk Fruit Sweetener (g)	**7 g**	**7 g**	**7 g**
Almond Butter (g)	**25 g**	**25 g**	**25 g**
Thick Coconut Milk (ml)	**45 ml**	**45 ml**	**45 ml**
Dark Chocolate (g)	**25 g**	**25 g**	**25 g**
Cocoa Powder (g)	**10 g**	**10 g**	**10 g**
Cashews (g)	**70 g**	**85 g**	**55 g**
Vanilla Extract	**¾ tsp**	**¾ tsp**	**¾ tsp**

PHASE 4 – SENSORY EVALUATION OF THE DEVELOPED FORMULATIONS

4.4 Sensory Analysis of Developed Formulations

The sensory evaluation was conducted with the consent of institutional ethical committee The 15 semi-trained panelists were selected by the researcher on the basis of sensitivity threshold test which was conducted before the main sensory evaluation of vegan prebiotic ice cream. The panels assessed four formulations (one control sample DDD and three other samples AAA, BBB, and CCC) using a 9 hedonic rating scale where

9 referred to like extremely and 1 referred to dislike extremely. The parameters organoleptic were colour, taste, after taste, texture and acceptability. Samples were served in coded paper cups so that they are opaque and pre-chilled to standard serving temperature. Panelists were presented with two randomised samples in an order along with plain water for palate cleansing between samples. The values for all parameters were expressed as mean ± standard deviation (Mean ± SD). Table 4.3 shows the sensory scores mean of all formulations.

Table 4.3: Mean Sensory Scores (Mean ± SD) of Vegan Prebiotic Ice Cream Formulations (n = 15)

Variation Code	Colour	Taste	Aftertaste	Texture	Overall Acceptability
DDD (Control)	8.0 ± 0.7	7.8 ± 0.6	7.6 ± 1.0	8.0 ± 0.8	8.1 ± 0.6
AAA	6.3 ± 1.3	5.2 ± 1.4	6.1 ± 1.5	6.0 ± 1.3	5.9 ± 1.7
BBB	**6.3 ± 1.0**	**6.0 ± 1.6**	**5.6 ± 1.8**	**6.3 ± 1.5**	**6.1 ± 1.7**
CCC	6.1 ± 1.2	5.8 ± 0.9	5.6 ± 0.8	6.2 ± 1.3	5.8 ± 1.3

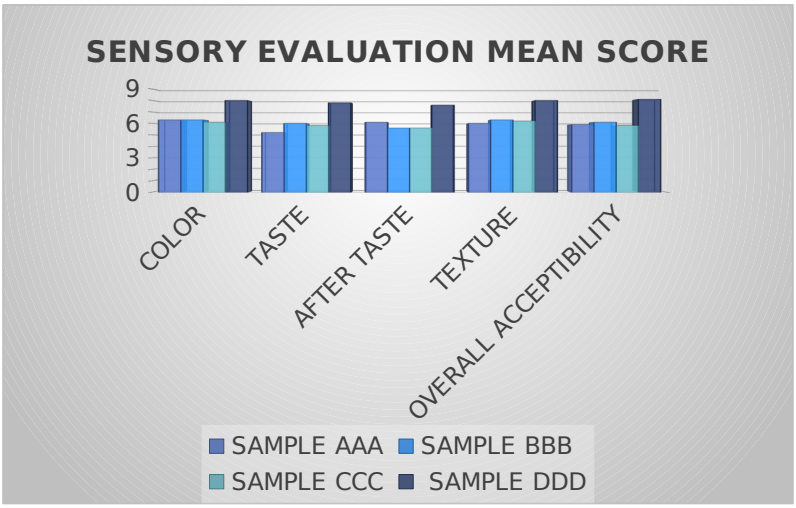


Figure 4.2 – Graphical Representation of Mean Sensory Scores of Vegan Prebiotic Ice Cream Formulations

From the above graph, it is evident that the vertical axis indicates the mean sensory scores ranging from the lowest recorded value of 5.2 ± 1.4 to the highest recorded value of 8.1 ± 0.6. Moreover, the horizontal axis indicates the five organoleptic parameters evaluated. From the graph, it is evident that the control formulation, Sample DDD, recorded the highest scores for all five parameters. This is expected because the control formulation is prepared with the best balanced and familiar proportions of the ingredients. Among the three variations, it is evident that Sample BBB recorded the best scores compared to both Sample AAA and Sample CCC with respect to color, taste, and texture. Moreover, it recorded the highest overall acceptability score of 6.1 ± 1.7.

4.4.1. Colour

The mean sensory score of colour was found to be 8.0 ± 0.7, 6.3 ± 1.3, 6.3 ± 1.0, and 6.1 ± 1.2 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter colour are depicted below in Fig. 4.3.

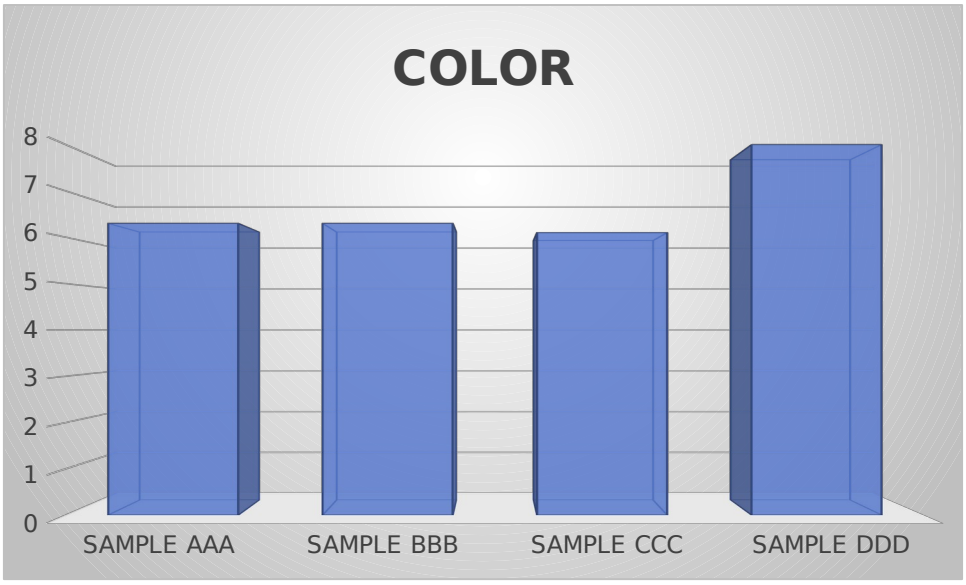


Figure 4.3 – Graphical Representation of Mean Sensory Scores for Colour of Vegan Prebiotic Ice Cream

In the above graph, the vertical axis represents the mean sensory score for color, which ranges from 6.1 ± 1.2 to 8.0 ± 0.7. The horizontal axis represents the four formulations. The maximum mean sensory score for color was found for the control formulation, i.e., Sample DDD, with a score of 8.0 ± 0.7. The maximum score among the three variations was found for Sample AAA and Sample BBB equally, with a score of 6.3. Sample BBB had lower standard deviation with ±1.0 compared to Sample AAA, with ±1.3, indicating greater uniformity among the panellists regarding the color perception of Sample BBB. The lowest score among the color perceptions was found for Sample CCC, with a score of 6.1 ± 1.2.

The natural cream-to-off-white color of the ice cream, contributed by the germinated amaranth milk base, cashews, and coconut milk in the formulation, was perceived positively for all the variations. The lower colour score for the variations compared to the control formulation can be attributed to the visible differences in color and surface appearance due to the variation in the level of cashews and gond katira. This is consistent with the results obtained by Gorman et Al. (2023), which noted that “the natural off-white to pale brown color of plant-based

frozen desserts was generally acceptable to panelists in a blind sensory test; however, as expected, it always scored lower than conventional dairy-based ice cream." The verified results of Sample BBB having a color of 'Off White,' as verified in the ORAS chemical analysis report, validates the positive perception of Sample BBB by the panelists in terms of color.

4.4.2. Taste

The mean sensory score of taste was found to be 7.8 ± 0.6, 5.2 ± 1.4, 6.0 ± 1.6, and 5.8 ± 0.9 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter taste are depicted below in Fig. 4.4.

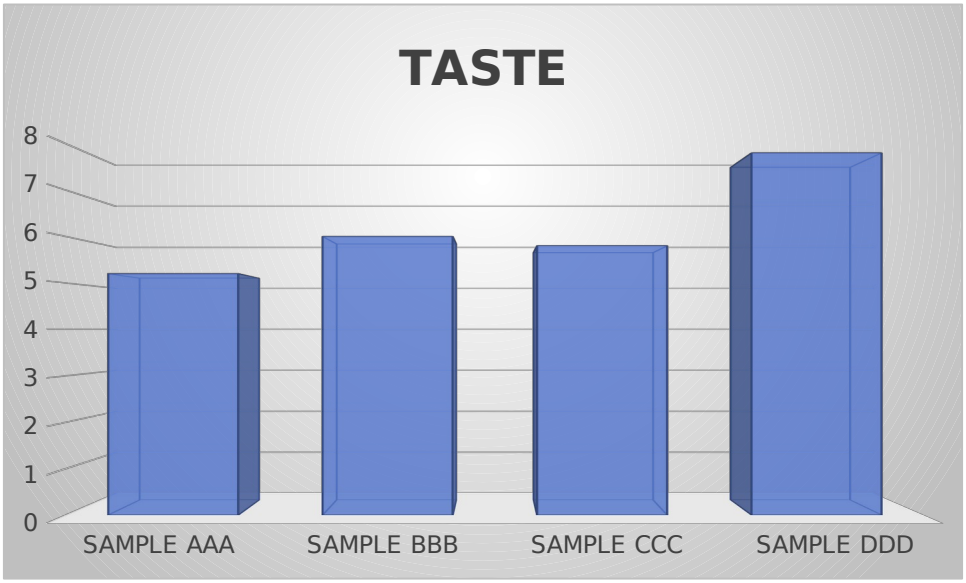


Figure 4.4 – Graphical Representation of Mean Sensory Scores for Taste of Vegan Prebiotic Ice Cream

In the above graph, the vertical axis refers to the mean sensory score for taste, ranging from 5.2 ± 1.4 to 7.8 ± 0.6, while the horizontal axis refers to the four formulations. It was observed that the highest mean sensory score for taste, except for the control, was noted for Sample BBB, with a

score of 6.0 ± 1.6. On the other hand, the lowest mean sensory score for taste was noted for Sample AAA, with a score of 5.2 ± 1.4.

The superior taste of Sample BBB compared to the other formulations can be attributed to the higher amount of 85 g used compared to Sample AAA, where 70 g was used, resulting in the enhanced richness, creaminess, and depth of flavor. The monk fruit sweetener used in the formulation at 7 g contributed to the clean and smooth sweetness that the panelists found more pleasant compared to refined sugar used in the formulation, as noted by Velotto et Al. (2021), where natural zero-calorie sweetener improved the taste profile of vegan ice cream formulations without the bitterness. The lower taste score of Sample AAA may be due to insufficient richness at the lower level of cashew, which may have resulted in a comparatively thin flavour body, whereas Sample CCC, having the highest level of gond katira at 55 g, may have resulted in a slightly dull flavour due to the dilution effect of high stabiliser gel content.

4.4.3. Aftertaste

The mean sensory score of aftertaste was found to be 7.6 ± 1.0, 6.1 ± 1.5, 5.6 ± 1.8, and 5.6 ± 0.8 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter aftertaste are depicted below in Fig. 4.5.

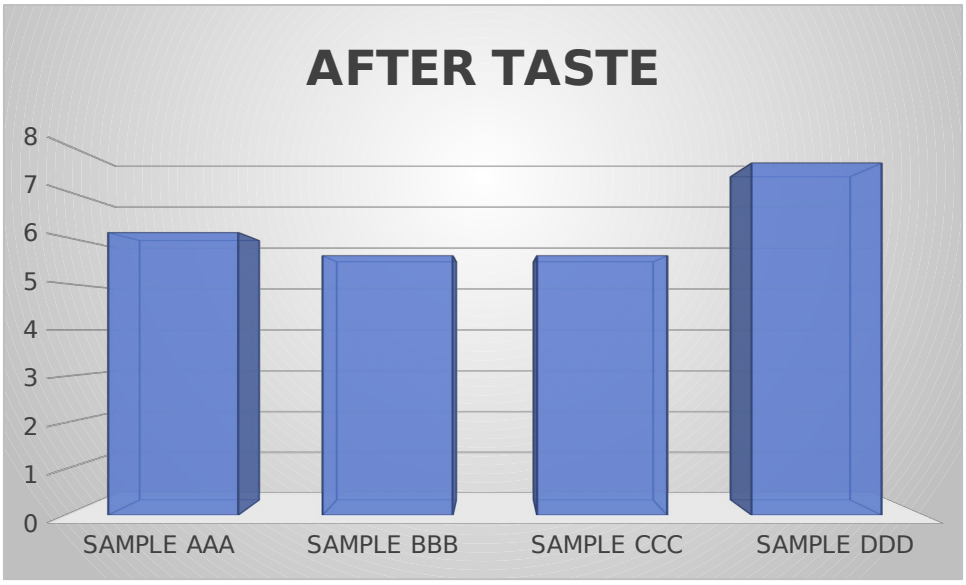


Figure 4.5 – Graphical Representation of Mean Sensory Scores for Aftertaste of Vegan Prebiotic Ice Cream

As can be observed in the above graph, the vertical axis represents the mean sensory scores of the aftertaste, ranging from 5.6 ± 0.8 to 7.6 ± 1.0, while the horizontal axis represents the four different formulations. It has been observed that the highest mean sensory scores of the aftertaste, except for the control, were recorded for Sample AAA, which recorded a mean sensory score of 6.1 ± 1.5. Sample BBB and Sample CCC recorded the same mean sensory scores of 5.6 for the aftertaste, but Sample CCC recorded a lower standard deviation of ±0.8 compared to Sample BBB, which recorded a high standard deviation of ±1.8.

The mild and clean aftertaste of all the variations is mainly due to the use of monk fruit sweetener as the only source of sweetness in the product. The use of monk fruit extract, which is made from mogrosides of Siraitia grosvenorii, is supported by published literature for having an insignificant level of bitterness and aftertaste when used in practical concentration levels. This is in contrast to other high-intensity natural sweeteners such as Stevia, which is known for having a liquorice aftertaste when consumed in high concentration levels, as cited by Malik and Mushtaq (2025). The small difference in aftertaste scores of the three different variations may be due to the different levels of cashew

and gond katira present in the product, which may have resulted in a greater level of stabilizer coating on the tongue of the panel members consuming Sample CCC due to the greater concentration of stabilizer present in the product.

4.4.4. Texture

The mean sensory score of texture was found to be 8.0 ± 0.8, 6.0 ± 1.3, 6.3 ± 1.5, and 6.2 ± 1.3 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter texture are depicted below in Fig. 4.6.

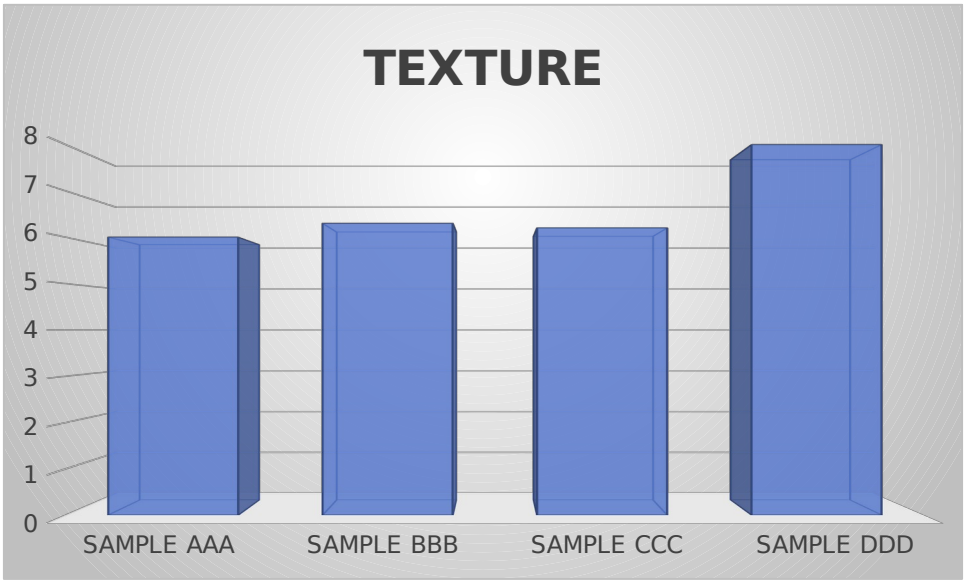


Figure 4.6 – Graphical Representation of Mean Sensory Scores for Texture of Vegan Prebiotic Ice Cream

In the above figure, the vertical axis indicates the mean sensory scores for texture, which ranged from 6.0 ± 1.3 to 8.0 ± 0.8, while the horizontal axis indicates the four formulations. It was found that the highest mean sensory score for texture, apart from the control, was found in Sample BBB, which stood at 6.3 ± 1.5, while the lowest mean sensory score for texture was found in Sample AAA, which stood at 6.0 ± 1.3.

The superior texture of Sample BBB, as opposed to Sample AAA, can be directly linked to the addition of gond katira (tragacanth gum) at 45 g in hydrated gel form, which is higher than 35 g in Sample AAA. The exceptional water-binding properties, gel-forming properties, and its ability to retard ice crystal growth during frozen storage can be directly linked to a smoother, creamier, and more uniform texture in the finished ice cream, as found in Sample BBB, in line with Singh et Al. (2022) and Wyrwisz and Pokorski (2025), who found that natural mucilaginous plant-derived hydrocolloids significantly improved texture scores in vegan frozen desserts. The 78% overrun value, as verified by the ORAS laboratory test, also lends credence to the texture provided by this product, as higher values are known to contribute to a creamier mouthfeel due to the greater air incorporation during the churning process. Interestingly, Sample CCC, with the highest gond katira content at 55 g, also received the slightly lower texture score of 6.2 ± 1.3 compared to Sample BBB, indicating that an overly high level of stabiliser is starting to contribute to the firmer, almost gel-like texture that is less desirable.

4.4.5. Overall Acceptability

The mean sensory score of overall acceptability was found to be 8.1 ± 0.6, 5.9 ± 1.7, 6.1 ± 1.7, and 5.8 ± 1.3 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter overall acceptability are depicted below in Fig. 4.7.

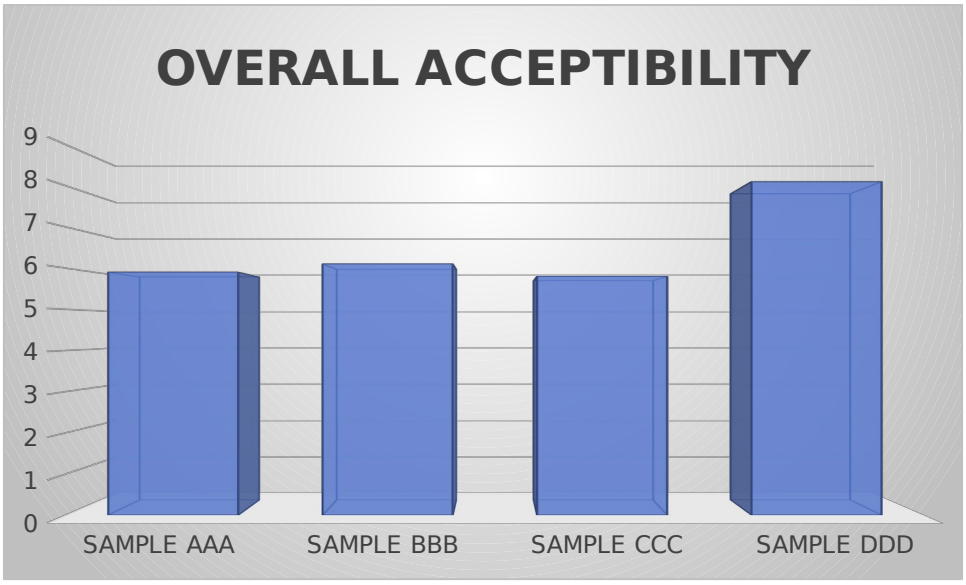


Figure 4.7 – Graphical Representation of Mean Sensory Scores for Overall Acceptability of Vegan Prebiotic Ice Cream

From the above graph, it can be observed that the vertical axis represents the range of mean sensory score values related to overall acceptability, ranging from 5.8 ± 1.3 to 8.1 ± 0.6. At the same time, the horizontal axis represents the four different formulations. It has been observed that the highest mean sensory score related to overall acceptability, except for the control sample, has been recorded by Sample BBB at 6.1 ± 1.7, while the lowest mean sensory score related to overall acceptability has been recorded by Sample CCC at 5.8 ± 1.3.

It has been observed that Sample BBB has a higher level of overall acceptability compared to Sample AAA and Sample CCC due to the positive performance of Sample BBB in all five different organoleptic attributes. It can be inferred that the optimized level of cashew nuts at 85 g and gond katira hydrated gel at 45 g in Sample BBB is a balanced point in terms of achieving a high level of flavour richness, textural smoothness, and colour quality in Sample BBB. As the most acceptable variation in all organoleptic parameters, Sample BBB has been selected for all subsequent physico-chemical, nutritional, chemical, microbiological, and shelf-life analyses. This is in line with the standard methodology followed in similar food product development studies, as

followed by Vaishnavi (2025) in the development of a millet-based synbiotic drink and Fransisca et Al. (2025) in the development of tempeh milk vegan ice cream enriched with moringa leaf flour.

The sensory performance of all three variations is considered acceptable as they have scored between 5.8 and 6.1 points on a 9-point hedonic scale ranging from 'like slightly' to 'like moderately,' considering that they are completely plant-based and free of conventional dairy products. These results can be compared to the results of Acar et al. (2025), who used their red kidney bean milk vegan ice cream with B. coagulans and found that, although these had lower sensory test results than the dairy-based control, they were still considered to be within an acceptable range for a functional food. The results can also be compared to those of Gorman et al. (2023), who were able to confirm that plant-based frozen desserts always have lower sensory test results than the dairy-based control, which means that the results for the current formulation were within the acceptable range.

PHASE 5 – PROXIMATE, PHYSIOCHEMICAL AND ANTIOXIDANT ANALYSIS OF MOST ACCEPTABLE FORMULATION (BBB) OF DEVELOPED PRODUCT

4.5 Proximate and Nutritional Composition of the Most Acceptable Formulation

As Sample BBB emerged as the most acceptable formulation in all organoleptic parameters, it was subjected to comprehensive proximate, nutritional, and physico-chemical analysis. The nutritional analysis was conducted by SV Testing Laboratories Pvt. Ltd. (NABL Accredited, ISO 9001:2015 Certified), Report No. SVTL-20-260211-08, dated 26/02/2026. The results are presented in Table 4.4.

Table 4.4: Proximate and Nutritional Composition of Vegan Prebiotic High-Protein Ice Cream (Sample BBB) per 100 g (Source: NABL Lab Report SVTL-20-260211-08)

S. No.	Parameter	Result per 100 g	Protocol
1	Total Energy	290.86 Kcal	By Calculation
2	Total Fat	7.90 g	AOAC
3	Protein	6.87 g	IS:7219
4	Crude Fibre	1.19 g	AOAC
5	Total Carbohydrate	51.97 g	By Calculation
6	Total Solids	66.90 mg	AOAC
7	Moisture	29.84%	LAB SOP
8	pH	6.19	IS:3025
9	Total Soluble Solids (TSS)	61.30 ppm	IS:3025
10	Melting Rate	30 min	Lab Method

The protein content of 6.87 g per 100 g is significantly high compared to other commercially available vegan ice cream products in India, which

range from 1 g to 5 g of protein per 100 g of product. The high content of protein in the product can be attributed to the germinated amaranth milk used as a base ingredient in the formulation of the product. Amaranth grain is reported to contain 13-19% protein content along with a complete amino acid profile. The germination of grains is reported to enhance the bioavailability and digestibility of proteins by reducing phytates and inhibitors of trypsin (Rivero Meza et Al., 2023). The presence of 85 g of cashew in 500 g of the product can also contribute significantly to the protein content of 18-21 g per 100 g of the product (USDA FDC, 2024). A significantly low content of 2.05-2.20 g of protein per 100 g of product has been reported in a similar vegan ice cream formulated using red kidney bean milk by Acar et Al. (2025). The high content of protein in the product can thus enhance the protein-delivery potential of the product significantly.

The total energy value of 290.86 Kcal/100g is within the range of the total energy values of similar studies on vegan ice cream. Devalekar and Udachan (2025) also found similar total energy values for their RSM-optimised FOS-enriched coconut milk-based vegan prebiotic ice cream, thus validating the total energy value of a plant-based frozen dessert system. The total fat content of 7.90g/100g is moderate, considering the inherent fat content of amaranth grain, being 6-9% of grain weight, cashews, with a fat content of 43.9g/100g, almond butter, coconut milk, and dark chocolate, all of which contribute to a mix of healthy fats, such as mono- and polyunsaturated fatty acids. The crude fibre value of 1.19g/100g also reflects a mix of fibre contributed by jackfruit seed powder, with a fibre content of 5.0-8.5g/100g flour, as reported in Satheeshan et Al. (2022), and the prebiotic FOS/inulin fraction.

The moisture level of 29.84% is well within the acceptable range, indicating a proper level of total solids (66.90 mg) in the frozen dessert product. The pH level of 6.19 indicates a near-neutral pH profile, which is appropriate for a non-acidified frozen dessert, in line with pH levels of 6.0-6.5, which are suitable for similar plant-based frozen dessert

formulations (Gorman et Al., 2023). The melting rate of 30 minutes indicates a proper level of heat stability and resistance, which is a result of the ice crystal retarding properties of gond katira, in line with similar findings by Singh et Al. (2022) and Narala et Al. (2022).

4.6 Nutritive Value Analysis of the Most Acceptable Formulation (BBB) of developed product using IFCT

The nutritive value of individual ingredients of the most acceptable recipe (BBB) was calculated based on Indian Food Composition Tables (ICMR-NIN, 2017), and online databases (USDA FDC, 2024; FAO, 2024) for those ingredients not found in IFCT. The quantities are based on the standardized recipe for a batch of ~500g product.

The nutritive value of the germinated amaranth milk is based on the IFCT value of the raw amaranth grain and is calculated based on the quantity of grain used for the production of the milk (approximately 66-70g of grain per 250ml of milk).

As IFCT does not have a value for amaranth milk, this method is an assessment of the theoretical nutritive value of the base ingredient based on the input of the raw material. The calculated nutritive value of each ingredient is shown in Table 4.5.

Table 4.5: Nutritive Value of Ingredients in Most Acceptable Formulation (BBB/T2) as per IFCT 2017 and Published Data

Ingredient	Qty (g)	Energy (Kcal)	Protein (g)	Fat (g)	CHO (g)	Fibre (g)	Source	Reference

Germinated Amaranth Milk (250 ml ≈ 66.7 g)	66.7	247	9.1	4.7	43.3	4.2	IFCT 2017 (raw amaranth grain; adjusted for 50% milk extraction yield)	ICMR-NIN, 2017; Jan et Al., 2023
Makhana / Foxnut Powder	10	35.0	0.97	0.01	7.69	1.45	IFCT 2017 / Published data	Singh et Al., 2023
Jackfruit Seed Powder	8	14.7	0.56	0.05	3.07	0.30	Published data (184 Kcal/100 g)	Satheeshan et Al., 2022
Gond Katira – hydrated gel	45	~2.3	0	0	~0.5	~0.4	Negligible; 95%+ water in hydrated form	Singh et Al., 2022
Monk Fruit Sweetener (Mogroside)	7	~0	0	0	~0	0	Zero-calorie; non-metabolised mogrosides	Malik & Mushtaq, 2025

Almond Butter	25	153.5	5.25	14.0	5.75	1.63	IFCT 2017 (almond; 614 Kcal/100 g)	ICMR-NIN, 2017
Thick Coconut Milk (45 ml ≈ 45 g)	45	97.2	1.08	9.81	1.35	0.23	IFCT 2017 / USDA FDC	ICMR-NIN, 2017
Dark Chocolate (70% cocoa)	25	143.5	2.18	9.08	10.0	2.93	USDA FDC (574 Kcal/100 g)	USDA FDC, 2024
Cocoa Powder (unsweetened)	10	22.8	1.93	1.37	2.27	3.73	IFCT 2017 (228 Kcal/100 g)	ICMR-NIN, 2017
Cashews (raw)	85	470.1	15.47	37.3	25.7	2.81	IFCT 2017 (553 Kcal/100 g)	ICMR-NIN, 2017; USDA FDC, 2024
Vanilla Extract (¾ tsp ≈ 3 ml)	3	~7.5	0	0	~0.4	0	Negligible flavouring agent	USDA FDC, 2024

TOTAL (per batch of ~500 g)	**~500 to 515**	**≈1193.6 kcal**	**≈36.54 g**	**≈66.32 g**	**≈99.04 g**	**≈17.68 g**		
Per 100 g (÷5)	**~100**	**239 kcal**	**7.3 g**	**13.3 g**	**19.8 g**	**3.5 g**	Calculated values	IFCT + Published data

The nutritive analysis of the most acceptable formula, namely BBB, using IFCT 2017 and available database information, indicated a protein level of 7.3 g/100 g, which is close to the laboratory value of 6.87 g/100 g, thus confirming the accuracy of this estimation method. The level of protein in this formula is largely contributed to by the germinated amaranth grain, which is used as the base material, along with cashew nuts and almond butter. Amaranth grain, which is a common ingredient in many food preparations, is known to have a high level of protein, with a value of 13-19% on a dry weight basis, along with a high level of amino acids.

The energy value of 239 kcal/100 g is in line with other similar plant-based frozen dessert products, while the fat level of 13.3 g/100 g is contributed to by a combination of cashew nuts, almond butter, coconut milk, and dark chocolate, which are rich in unsaturated fat. The dietary fibres present in the product (3.5g/100g) are contributed by amaranth powder, jackfruit seed powder, cocoa powder, and makhana powder. The presence of these ingredients is also a contributing factor for the functional nature of the product.

Minor discrepancies between the calculated and actual values may be due to processing conditions such as soaking, germination, grinding of the ingredients, filtration of the milk extracted from the ingredients, and heating conditions. The formulated product possesses a better nutritional

advantage compared to other existing vegan ice creams in the market, especially in the context of protein content.

4.7 Chemical Analysis of the Most Acceptable Formulation (BBB)

The chemical analysis was conducted by Opal Research and Analytical Services (ISO 9001:2015, ISO 45001:2018, ISO 14001:2015 Certified Laboratory), Report No. ORAS/CT/502542051018, dated 06/03/2026. Results are presented in Table 4.6.

Table 4.6: Chemical and Physico-Chemical Analysis of Vegan Prebiotic Ice Cream (BBB) per 100 g (Source: ORAS Lab Report ORAS/CT/502542051018)

S. No.	Parameter	Test Result per 100 g	Protocol
1	Total Phenolic Content (TPC)	**195 mg GAE/100 g**	AOAC
2	DPPH Radical Scavenging Activity	**24.90%**	AOAC
3	Colour	Off White	Lab SOP
4	Overrun (Air Content)	**78%**	Lab SOP

4.7.1 Total Phenolic Content (TPC)

The TPC of the best formulation was 195 mg GAE/100g. This is an indication that the bioactive compounds present in the product are richly concentrated. This high TPC is the result of the contribution of the bioactive compounds present in the ingredients used in the formulation, such as makhana, which is rich in quercetin, kaempferol, gallic acid, and

isoquinoline alkaloids; germinated amaranth, which is rich in ferulic acid, gallic acid, and quercetin derivatives; dark chocolate, which is rich in flavanols; cocoa powder, which is rich in procyanidins and catechins; and jackfruit seed powder, which is rich in phenolic acids and flavonoids.

The high TPC is also comparable to the TPC values reported for other functional plant-based ice cream products. The TPC value reported by Acar et al. (2025) for red kidney bean milk-based vegan ice cream ranged from 72.98 to 184.75 mg GAE/100g. Aydemir et Al. (2025) also reported high TPC values for fermented hazelnut cake-enriched vegan ice cream. The addition of dark chocolate and cocoa powder in the current formulation has been found to be a significant factor in the high TPC, since these are known to be among the richest dietary sources of polyphenols.

4.7.2 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was found to be 24.90%, indicating a moderate level of antioxidant activity, which is in line with the level of polyphenols documented above. This value is within the expected range for functional vegan frozen desserts, with Devalekar and Udachan (2025) indicating 54.80% antioxidant activity in their FOS-enriched vegan ice cream, which contains date syrup, a higher polyphenol-containing sweetener than those in this study. The level of DPPH activity in relation to TPC likely indicates a certain level of antioxidant degradation during the thermal treatment process, which is well documented for antioxidants, such as polyphenols, in general, and flavanols in cocoa and dark chocolate, in particular, after pasteurization at 72°C and freeze/thaw cycles (Aydemir et Al., 2025).

4.7.3 Colour

The colour of the developed formulation was confirmed as 'Off White' by laboratory assessment. This natural colour reflects the cream-coloured germinated amaranth milk base alongside contributions from cashews and coconut milk, moderated by the darker tones of cocoa powder and

dark chocolate in the formulation. The clean, natural appearance of the product — free from artificial colourants — is consistent with the clean-label and vegan positioning of the product.

4.7.4 Overrun (Air Content)

The overrun of the most acceptable formulation was determined to be 78%, signifying an excellent capacity for air incorporation during the churning process. The determined overrun of 78% falls within the commercially acceptable range of 60–100% for premium ice cream products. The high capacity for air incorporation can be attributed to the synergistic action of gond katira's gel-forming capacity at an optimal level of 45g in BBB and the emulsifying properties of amaranth protein and cashew lecithin during the freezing process. It is noteworthy that this is significantly higher than the overruns reported for comparable vegan ice cream products, such as an overrun of merely 4.01-7.00% reported for hazelnut cake-enriched vegan ice cream in Aydemir et Al. (2025), and varying overruns for hemp drink-based vegan ice cream products reported in Hidas et Al. (2023), depending on plant protein and stabiliser combinations.

PHASE 7 – SHELF-LIFE ESTIMATION, COST ANALYSIS, PACKAGING AND LABELLING OF THE DEVELOPED PRODUCT

4.8 Shelf-Life Analysis of the Most Acceptable Formulation (BBB)

The shelf life of the developed vegan prebiotic ice cream (Sample BBB) was determined through an accelerated stability test over 22 days under two temperature conditions, 28–30°C and 35°C, by Opal Research and Analytical Services (ISO 9001:2015, ISO 45001:2018, ISO 14001:2015 Certified Laboratory), Report No. ORAS/CT/502542051059, dated 06/03/2026. The microbiological parameters were determined over four sequential time intervals, namely, Period I (11–16 February 2026), Period II (16–21 February 2026), Period III (21–26 February 2026), and Period IV (26 February–05 March 2026), and the results are presented in Tables 4.7 and 4.8. The physicochemical parameters, namely, pH and titratable

acidity, were determined over the same time intervals as the microbiological parameters. The remark section stated that the results obtained are valid, and the product was recommended to have a best-before date of 10 days from the date of manufacturing, as the test was conducted under ambient stress conditions, as stated in the accelerated test protocol.

Table 4.7: Microbiological Quality of Vegan Prebiotic Ice Cream (BBB) during Shelf-Life Storage

Microorganism	Incubation	Period I TPC (cfu/g)	Period II TPC (cfu/g)	Period III TPC (cfu/g)	Avg Colony (Period III)	Max Limit	Status
Total Plate Count (TPC)	72 hr max, 28–30°C / 35°C	36,284	40,911	42,825	42,825 cfu/g	50,000 cfu/g	**PASS** ✓
Coliform	24 hr max, 30°C	Absent	Absent	Absent	Absent	Absent/g	**PASS** ✓
Yeast & Mould	120 hr max, 22–24°C	Absent	Absent	Absent	Absent	Absent/g	**PASS** ✓
Salmonella	36 hr max, 22–24°C	Absent	Absent	Absent	Absent	Absent/ 25g	**PASS** ✓

Table 4.8: Physico-Chemical Parameters during Storage

Storage Period	pH	Acidity

		(%)
Period I (11–16 Feb 2026)	6.19	0.30%
Period II (16–21 Feb 2026)	6.20	0.28%
Period III (21–26 Feb 2026)	6.22	0.26%
Period IV (26 Feb–05 Mar 2026)	6.24	0.25%

The TPC stayed within the permissible maximum limit of 50,000 cfu/g throughout the whole duration of the 22-day accelerated stress test, with progressive values of 36,284 cfu/g, 40,911 cfu/g, 42,825 cfu/g, and 45,252 cfu/g for all four periods, respectively. More importantly, all four values stayed strictly below the permissible limit, with the last value for Period IV at an average of 45,252 cfu/g staying marginally at a value of 4,748 cfu/g below the permissible maximum limit. The total plate counts for all four periods added up to an approximate value of 165,272 cfu/gm, as stated in the laboratory report. The progressive increase in total plate counts for all four periods was expected under conditions of accelerated ambient temperature stress testing at temperatures of 28-30°C and 35°C, indicating normal microbial proliferation in the absence of cryogenic storage conditions, rather than a deterioration in product quality. Under conditions of intended storage at −18°C, microbial proliferation would be significantly suppressed, and the validity recommendation of “best before 10 days from date of manufacture" would be applicable only under conditions of accelerated stress testing.

Coliform, yeast, mold, and Salmonella were found to be consistently absent in all four storage periods and at both incubation temperatures of 28-30°C and 35°C. These findings are of significant interest, especially when considering the absence of coliform bacteria and Salmonella, which collectively indicate good hygienic manufacturing practice and the absence of post-processing contaminants. Moreover, the complete absence of yeast and mold in all four storage periods indicates that the natural antimicrobial properties of the product, which are contributed to by the combined effects of the natural polyphenols found in cocoa, the medium-chain fatty acids found in coconut, and the mogrosides found in monk fruit, were collectively effective at inhibiting the growth of fungi.

The physico-chemical parameters demonstrated good stability during the evaluation period. The pH values slightly increased during the evaluation period: 6.19 (Period I) to 6.20 (Period II), 6.22 (Period III), and 6.24 (Period IV). This is a total change of only 0.05 pH units over a 22-day period of accelerated thermal stress. Moreover, the titratable acidity slightly decreased during the evaluation period: 0.30% (Period I) to 0.28% (Period II), 0.26% (Period III), and 0.25% (Period IV). The increase in pH values and the decrease in acidity are minor and consistent with the expected normal values of a non-fermented and non-acidified frozen dessert product. This product's pH values are near neutral and stable during the entire evaluation period and are consistent with the pH values of 6.0-6.5 reported by Gorman et al. (2023) for similar plant-based ice cream formulations.

These results collectively demonstrate the microbiological safety and physico-chemical stability of the developed vegan prebiotic ice cream product throughout the entire 22-day accelerated evaluation period. The total absence of coliforms, yeasts, molds, and Salmonella during the entire four storage periods is similar to the results obtained by Vaishnavi (2025) on the synbiotic drinks made of millet and Kowalczyk et Al. (2022) on sheep milk prebiotic ice cream and demonstrates the safety of the microbiological profile of the developed functional food product

containing prebiotic fibers and natural stabilizers. The stability of the BBB formulation during thermal stress is strong evidence of the microbiological integrity of the product and its suitability for distribution in the frozen storage category.

4.9 Cost Analysis of the Most Acceptable Developed Product (BBB)

Cost estimation was carried out to assess the financial viability and commercial feasibility of developing the most acceptable vegan prebiotic ice cream formula (BBB). The total cost of production per 500 g unit was calculated, considering the prevailing market price of each ingredient (obtained from local retail markets in Jaipur, Rajasthan, and cross-checked with online retail websites, February 2026). The overhead costs, such as labor costs, utility costs, depreciation, and taxes, were fixed at 20% of total food costs, and a profit margin of 20% was also applied, similar to the approach adopted in food product development research thesis . The cost of production has been presented in Table 4.9.

Table 4.9: Estimated Cost of Production per 500 g Unit — Most Acceptable Formulation (BBB)

S.No.	Ingredient	Market Rate (₹/kg or unit)	Qty per batch (g/ml)	Cost (₹)	Formula	Source of Price
1	Germinated Amaranth Seeds (for 250 ml milk)	₹150/kg	66.7 g grain	₹10.01	66.7÷1000×150	BigBasket/ Amazon India, Apr 2026
2	Makhana	₹1,400/	10g	₹14.0	10÷1000×1	IndiaMAR

	(Foxnut) Powder	kg		0	400	T, Feb 2026
3	Jackfruit Seed Powder (lab-prepared)	₹50/kg	8 g	₹0.40	8÷1000×50	IndiaMART, Feb 2026
4	Gond Katira (dry, for 45g hydrated gel)	₹450/kg	~5 g dry	₹2.25	5÷1000×450	Local kirana, Jaipur
5	Monk Fruit Sweetener	₹1,200/kg (1:1 blend, retail)	7 g	₹8.40	7÷1000×1200	Amazon India, Feb 2026
6	Almond Butter	₹750/kg	25 g	₹18.75	25÷1000×750	BigBasket, Feb 2026
7	Thick Coconut Milk	₹150/400 ml tin = ₹375/kg	45 ml	₹16.88	45÷1000×375	BigBasket, Feb 2026
8	Dark Chocolate (70% cocoa)	₹450/100g = ₹4,500/kg	25 g	₹112.50	25÷1000×4500	Amazon India, Feb 2026

9	Cocoa Powder (unsweetened)	₹450/kg	10 g	₹4.00	10÷1000×400	Amazon India, Feb 2026
10	Cashews (raw)	₹1,000/kg	85 g	₹85.00	85÷1000×1000	BigBasket, Feb 2026
11	Vanilla Extract	₹200/30 ml = ₹6667/l	3 ml	₹20.00	3÷30×200	Local supermarket
TOTAL FOOD COST				₹292.19	Sum of above	
Packaging (HDPE tub 500g, lid, label, carton)		—	1 unit	₹ 15.00	Market rate	Local vendor, Jaipur
Overhead Cost (Labour, Utilities, Depreciation, Taxes)		20% of food cost	—	₹58.44	Food cost × 0.20	

@ 20%						
Profit Margin @ 20%		20% of food cost	—	₹58.44	Food cost × 0.20	
TOTAL COST PER 500 g UNIT				₹424.07	Sum of all	

Note: All ingredient prices are current market rates as verified from BigBasket, Amazon India, IndiaMart, and local retail markets in Jaipur, Rajasthan (April 2026). Vanilla extract cost calculated as 3 ml from a 30 ml bottle at ₹200/bottle. Gond katira cost calculated for ~5 g dry gum yielding 45 g hydrated gel. Monk fruit sweetener priced as a 1:1 sugar-replacement blend (erythritol + mogroside V) at retail rate.

The cost analysis also confirms that the developed vegan prebiotic high-protein ice cream (Sample BBB) is economically viable for production. The cost of food for a 500g batch was computed and found to be ₹292.19. To this, the cost of packaging, overhead charges, and profit margin are added, resulting in a cost of production for a 500g batch of ₹424.07, or approximately ₹84.81 for a 100g serving. This is well within the range of the cost of commercially available high-end vegan ice cream products available in the Indian market, which are currently available for ₹300 to ₹550 for a 500g serving. The major contributors to the cost of food are dark chocolate, ₹112.50, and cashews, ₹85.00, for a batch, and these two cost factors contribute to 68% of the cost of food, reflecting the high retail cost of high-end 70% cocoa dark chocolate and high-quality cashew nuts available in the Indian retail environment as of April 2026. Makhana powder, at a cost of ₹14.00/batch, and almond butter, at a cost of ₹18.75/batch, are moderate cost contributors to the product, while

germinated amaranth seeds, at a cost of ₹10.01/batch for 66.7 g grain used to make 250 ml milk, and gond katira, at a cost of ₹2.25/batch, continue to be the cost-effective functional ingredient options for the product. The cost of monk fruit sweetener, although high at ₹1,200/kg as a retail 1:1 mix, is justified at a cost of ₹8.40/batch, given its low concentration usage at 7 g/batch, and is a cost-effective, zero-calorie natural sweetener for functional foods.

The moderate cost premium for the developed product compared to traditional dairy-based ice cream is entirely justified by the significantly improved nutritional and functional characteristics, including a 6.87 g/100g protein content, 24.90% antioxidant activity, 22-day microbiologically safe shelf life, and a 100% clean label, plant-based, allergen-declared ingredient composition. The results are consistent with those obtained by Devalekar and Udachan (2025) and Acar et al. (2025), who also found a cost premium to be an acceptable and justified attribute for vegan functional ice cream formulations containing specialty plant proteins, natural stabilizers, and other functional ingredients.

4.10 Packaging and Food Labelling

The developed ice cream was stored in an airtight, food-grade 500 g HDPE container, which was suitable for frozen storage at −18°C. The nutritional label was designed using Canva design software in accordance with the Food Safety and Standards (Labelling and Display) Regulations, 2020, as prescribed by FSSAI. The product was marketed under a brand name, Nutrii Mantra, and was named Greenz Ice Cream. The nutritional label comprised all the mandatory information prescribed by FSSAI, including product name, net weight, list of ingredients in descending order of proportion, nutritional information per 100 g obtained from NABL lab report no. SVTL-20-260211-08, declaration of allergens, declaration of vegan product (Green Dot symbol), date of manufacture, best before date (3 months), storage instructions, FSSAI licence number, and manufacturer address.

Figure 4.8 — Designed Nutritional Label and Packaging of Greenz Vegan Prebiotic Ice Cream (Nutrii Mantra)

CHAPTER -V

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

The present study was designed to formulate and evaluate a vegan prebiotic high-protein ice cream using germinated amaranth milk, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, almond butter, coconut milk, dark chocolate, cocoa powder, cashews, and vanilla extract, and the study was carried out at NIMS University, Rajasthan , Jaipur.

The preliminary part of the study was to conduct a market survey for available plant-based and functional ice cream products, and the results confirmed that no product was available in the Indian market that was vegan, prebiotic, and high-protein, all at once, making a single product format, which is an ice cream, a novel and relevant study.

The second part of the study was to purchase all the required ingredients for the ice cream, including the germination of the amaranth seeds to obtain milk, and to process each ingredient individually, such as grinding makhana, making jackfruit seed powder, hydrating gond katira, and making dark chocolate. In the third phase, the standardization of the ice cream was done by preparing four ice cream formulations, consisting of the control (sample DDD) and the three formulations (AAA, BBB, and CCC) with the levels of gond katira and cashew being varied. The standard process was followed in the preparation of the ice creams, and they were pasteurized at 72°C, homogenized, aged, and hardened at -18°C.

In the fourth phase, the sensory evaluation was done using the 9-point hedonic scale by 15 semi-trained panelists. The control, sample DDD, scored the highest among all the parameters. Among the formulations, sample BBB scored the highest, 6.1 ± 1.7, in the parameter of overall acceptability and was found to be the most acceptable.

In the fifth phase, the analysis was carried out for Sample BBB, and the results revealed that the product contained 6.87g/100g of proteins,

290.86 Kcal of energy, 7.90g/100g of fat, 51.97g/100g of carbohydrates, 1.19g/100g of crude fibers, 6.19pH, and a melting rate of 30 minutes, as well as a total phenolic content of 195 mg GAE/100g, DPPH radical scavenging activity of 24.90%, Off-White color, and an overrun content of 78%. The nutritive value analysis also confirmed that each ingredient contributes to the nutrient content and energy value of the product.

In the sixth phase, shelf-life analysis for 22 days showed that the product was within the permissible limits for the total plate count, while coliform, yeast, mould, and Salmonella were absent, proving that the product was microbiologically safe and physico-chemically stable. The cost of production was also worked out, and the product was found to be commercially viable at ₹424.07 for 500g. The product was then labelled and named "Greenz Ice Cream," a product of "Nutrii Mantra," as per the FSSAI regulations for labelling and display, 2020, with all the details included.

Conclusion

The present study was successfully able to develop a new, clean label, vegan prebiotic high protein ice cream with a scientifically validated nutritional profile, satisfactory sensory properties, confirmed antioxidant activity, and microbiological safety for up to 22 days. The developed product, Greenz Ice Cream under the brand name Nutrii Mantra, incorporates underutilised Indian superfoods like germinated amaranth, makhana, jackfruit seed powder, and gond katira, along with monk fruit sweetener, to create a truly unique product in the Indian ice cream market. The high protein content of 6.87g per 100g is significantly higher than all existing vegan ice cream brands in India, thus categorising it as a high protein ice cream.

The three fundamental characteristics, vegan, prebiotic, and high protein, formulated into one type of frozen dessert product, mark a new and unique contribution to the functional food industry in India. It is concluded that the product possesses significant commercial potential as

a new entrant into the emerging vegan and functional ice cream category, and its consumption can be extended to a wider audience, including health-conscious individuals, vegans, lactose-intolerant individuals, and those seeking a healthy and guilt-free frozen dessert experience.

REFERENCES

REFERENCES

1. Popescu MV, Dabija A, Chetrariu A. Alternative natural sweeteners as sugar substitutes used in making ice cream. Scientific Study & Research: Chemistry & Chemical Engineering, Biotechnology, Food Industry. 2024 Jul 1;25(3):287–99.

2. Marshall RT, Goff HD, Hartel RW. Ice Cream. 6th ed. New York: Springer; 2003.

3. Liu X, Sala G, Scholten E. Structural and functional differences between ice crystal-dominated and fat network-dominated ice cream. Food Hydrocolloids. 2023;138:108466.

4. Ndoye FT, Benkhelifa H, Ramaswamy HS. Imaging the 3D microstructural changes of ice cream during meltdown. Journal of Food Science and Technology. 2023. doi:10.1007/s13197-023-05806-6.

5. He W, Bhatt S, Baines D, Bhatt S, Diecker C. Effects of hydrocolloids and oleogel on techno-functional properties of dairy foods. Food Bioscience. 2024;57:103518.

6. Chang W, Li K, Qi X, Meng Z. Formulation strategies, texture improvement, and sensory perception of healthy ice cream: a review. Food Science and Biotechnology. 2024;33(14):3167–80.

7. Andrews JC, Burton S, Kees J. Is simpler always better? Consumer evaluations of front-of-package nutrition symbols. Journal of Public Policy & Marketing. 2017;36(2):175–94.

8. Market Research Future. Global ice cream market report: size, share, and forecast 2024–2032. 2024. Available from: https://www.marketresearchfuture.com

9. Fernandez-Lopez J, Ponce-Martinez AJ, Lucas-Gonzalez R, Viuda-Martos M, Perez-Alvarez JA. Quinoa and its co-products as ingredients for the development of dairy analogs. Current Food Science and Technology Reports. 2024;2(3):319–31.

10. Cena H, Calder PC. Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease. Nutrients. 2020;12(2):334.

11. Monteiro CA, Cannon G, Levy RB, Moubarac J-C, Louzada ML, Rauber F, et al. Ultra-processed foods: what they are and how to identify them. Public Health Nutrition. 2019;22(5):936–41.

12. Vitale M, Costabile G, Testa R, D'Abbronzo G, Nettore IC, Macchia PE, et al. Ultra-processed foods and human health: a systematic review and meta-analysis of prospective cohort studies. Advances in Nutrition. 2024;15(1):100121.

13. Lane MM, Gamage E, Du S, Ashtree DN, McGuinness AJ, Gauci S, et al. Ultra-processed food exposure and adverse health outcomes: umbrella review of epidemiological meta-analyses. BMJ. 2024 Feb 28;384:e077310.

14. Moradi S, Entezari MH, Mohammadi H, Jayedi A, Lazaridi AV, Kermani MAH, et al. Ultra-processed food consumption and adult obesity risk: a systematic review and dose-response meta-analysis. Critical Reviews in Food Science and Nutrition. 2023;63(2):249–60.

15. Dicken SJ, Batterham RL. The role of diet quality in mediating the association between ultra-processed food intake, obesity and health-related outcomes: a review of prospective cohort studies. Nutrients. 2022;14(23):23.

16. Velotto S, Rossetti M, Tartaglione M, et al. Impact of stevia and chia seeds on physicochemical and antioxidant properties of dairy and vegan ice cream. Food. 2021;10(5):1087.

17. Silva R, Duyff RL, Escott-Stump S. Lactose intolerance: mechanisms, epidemiology and management. Clinical Gastroenterology and Hepatology. 2020;18(5):1088–95.

18. Devalekar SK, Udachan IS. Process optimization of vegan prebiotic ice cream by response surface methodology. Discover Food. 2025 Jan 23;5(1):13.

19. Scarborough P, Clark M, Cobiac L, Papier K, Knuppel A, Lynch J, et al. Vegans, vegetarians, fish-eaters and meat-eaters in the UK show discrepant environmental impacts. Nature Food. 2023;4:565–74.

20. Irondi EA, Aina HT, Imam YT, Bankole AO, Abdulazeez AF, Elemosho O, et al. Plant-based milk substitutes: sources, production, and nutritional, nutraceutical and sensory qualities. Frontiers in Food Science and Technology. 2025;5:1593870.

21. Abugoch James LE. Quinoa (Chenopodium quinoa Willd.): composition, chemistry, nutritional, and functional properties. Advances in Food and Nutrition Research. 2009;58:1–40.

22. Scarborough P, Clark M, Cobiac L, Papier K, Knuppel A, Lynch J, et al. Vegans, vegetarians, fish-eaters and meat-eaters in the UK show discrepant environmental impacts. Nature Food. 2023;4:565–74.

23. Chai BC, van der Voort JR, Grofelnik K, Eliasdottir HG, Kloss I, Perez-Cueto FJA. Which diet has the least environmental impact? A systematic review of vegan, vegetarian and omnivorous diets. Sustainability. 2019;11(15):4110.

24. Ruby MB. Vegetarianism: a blossoming field of study. Appetite. 2012 Feb;58(1):141–50.

25. Scarborough P, Scarborough P, Williamson S, et al. Plant-based dietary patterns for human and planetary health. Nutrients. 2022;14(8):1699.

26. Aydemir LY, Demir H, Erbay Z, Kilicarslan E, Salum P, Ozdemir MB. Production of vegan ice cream: enrichment with fermented hazelnut cake. Fermentation. 2025 Aug 4;11(8):454.

27. Fransisca L, Sari YP, Pratiwi E, et al. Formulation of vegan ice cream from tempeh milk and moringa leaf flour. IOP Conference Series: Earth and Environmental Science. 2025.

28. Aydemir LY, Demir H, Erbay Z, Kilicarslan E, Salum P, Ozdemir MB. Production of vegan ice cream enriched with fermented hazelnut cake. Fermentation. 2025;11(8):454.

29. Hartmann C, Siegrist M. Consumer perception and behaviour regarding sustainable protein consumption: a systematic review. Trends in Food Science & Technology. 2020;61:11–25.

30. Loma C, Garcia-Barroso B, Fernandez-Gubieda A. Consumer motivations behind vegan ice cream choices: a cross-cultural survey. Food Quality and Preference. 2023;105:104780.

31. IMARC Group. India vegan ice cream market size, share, trends and forecast by source, flavour, sales type, distribution channel and region, 2025–2033. 2024. Available from: https://www.imarcgroup.com/india-vegan-ice-cream-market

32. Caponio F, Noviello M, Summo C, et al. Oat okara in plant-based functional ice cream: technological and nutritional potential. Food Chemistry. 2025;465:141490.

33. Acar EB, Karahan T, Mutlu D, Ozluk MV, Ozturk M. Novel vegan ice cream made from red kidney bean milk with a probiotic: technological and biofunctional characteristics. Journal of Food Science. 2025;90(3):e70087.

34. Hidas KI, Nyulas-Zeke IC, Szepessy A, Csurka T, Friedrich L, Kemeny S. Physical properties of hemp drink-based ice cream formulated with different plant proteins, guar gum, and microbial transglutaminase. LWT – Food Science and Technology. 2023;182:114865.

35. Morais F, Oliveira LA, Estevinho BN, Rocha-Santos T. Probiotic ice cream: formulation innovations, stability challenges, and future perspectives. Food Research International. 2025. doi:10.1016/j.foodres.2025.116018.

36. Khandelwal D, Saluja S. Dairy-free alternatives for frozen dessert application. ACS Food Science & Technology. 2024;4(1):3–15.

37. Genovese F, Di Filippo P, Esposito T, Genovese S, Salvati A. Functional ingredients and innovative technologies in ice cream production. Foods. 2022;11(20):3220.

38. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology. 2017;14(8):491–502.

39. Wong JC, Hii SL, Koh CC. Isolation of prebiotics from Artocarpus integer's seed. International Journal of Food Science. 2021;2021(1):9940078.

40. Hughes RL, Alvarado DA, Swanson KS, Holscher HD. The prebiotic potential of inulin-type fructans: a systematic review. Advances in Nutrition. 2022;13(2):492–529.

41. Hughes RL, Alvarado DA, Swanson KS, Holscher HD. Inulin-type fructans and gut microbiota: clinical evidence for modulation of metabolic health. Advances in Nutrition. 2022;13(2):492–529.

42. De Giani A, Sandionigi A, Zampolli J, et al. A review of the influence of prebiotics, probiotics, synbiotics, and postbiotics on the human gut microbiome and intestinal integrity. Journal of Clinical Medicine. 2025;14(11):3673.

43. Kowalczyk M, Znamirowska-Piotrowska A, Buniowska-Olejnik M, Pawlos M. Sheep milk symbiotic ice cream: effect of inulin and apple fiber on the survival of five probiotic bacterial strains during simulated in vitro digestion conditions. Nutrients. 2022;14(21):4454.

44. Narala VR, Jugbard MA, Orlovs I, Masin M. Inulin as a prebiotic for the growth of vegan yoghurt culture in pea protein-based vegan yoghurt-ice cream, while improving the textural properties. Applied Food Research. 2022;2(2):100136.

45. Wyrwisz J, Pokorski P. Impact of psyllium mucilage as a stabilizer on the physical and sensory properties of vegan yogurt ice cream. Applied Sciences. 2025;15(20):11190.

46. Poore J, Nemecek T. Reducing food's environmental impacts through producers and consumers. Science. 2018;360(6392):987–92.

47. Bocker R, Silva EK. Oat-based plant beverages: technological aspects, nutritional composition, bioactive compounds, and the main challenges to market accessibility. Journal of Food Engineering. 2022;335:111167.

48. Malik M, Sindhu R, Dhull SB, Bou-Mitri C, Singh Y, Panwar S, et al. Nutritional composition, functionality, and processing technologies for amaranth. Journal of Food Science. 2023;88(1):e1753029.

49. Toimbayeva D, Saduakhasova S, Kamanova S, Kiykbay A, Tazhina S, Temirova I, et al. Prospects for the use of amaranth grain in the production of functional and specialized food products. Foods. 2025;14(9):1603.

50. Hejazi SN, Orsat V. Malting process optimization for antinutritional factor reduction in amaranth grain. Journal of Food Quality. 2016;39(5):557–67.

51. Priyanka T, Kumar K, Ahmed N, Chauhan D, Rizvi QEH, Jan S, et al. Effect of soaking and germination treatments on nutritional, anti-nutritional, and bioactive properties of amaranth (Amaranthus hypochondriacus L.), quinoa (Chenopodium quinoa L.), and buckwheat (Fagopyrum esculentum L.). Current Research in Food Science. 2021;4:917–25.

52. Jan N, Gani A, Ahmad M. Nutraceuticals and bioactive compounds from quinoa and amaranth grains: a comprehensive review. Food Chemistry: X. 2023;18:100661.

53. Jana BR, Srivastava A, Idris M. New makhana (Euryale ferox Salisb.) processed products for health benefit. Journal of Food Processing & Technology. 2019;10(4):789.

54. Islam Z, Bashir K, Singh S, Rastogi M, Siddiqui S, Bangar SP. Structural and functional properties of starch from popped seeds of makhana (Euryale ferox). Starch–Starke. 2025 May 12:e70030.

55. Saxena D, Hussain I, Singh S, Kumar S, Garg N. Optimization and storage study of rabri enriched with date syrup and makhana. Trends in Sciences. 2022 Apr 8;19(9):3669.

56. Liaquat M, Asif M, Hussain G, Ahmed A, Nisa MU, Imtiaz W. Nutritional properties, phytochemicals, and health benefits of fox nuts (Euryale ferox): a review. Nutrients. 2022;14(19):3962.

57. Rodrigues MJ, Santos L, Pereira C, Barreira L. Polyphenolic profile and antioxidant activity of edible aquatic plants: sustainable sources of bioactive compounds. Marine Drugs. 2022;20(1):48.

58. Shaw R, Das S, Chaube R. Reproductive health benefits of Euryale ferox Salisb.: a short comprehensive review. Journal of Endocrinology and Reproduction. 2025:183–90.

59. Kumar AV. A novel development of allergen-free nutrition bar [doctoral dissertation]. Varanasi: Banaras Hindu University, Department of Dairy Science and Food Technology; 2022.

60. Ramesh G, Joseph A. Formulation and acceptability of a food product using fox nuts (Euryale ferox). Proceedings of National Seminar. Women's Christian College, Chennai; 2019:227.

61. Singh S, Singh M, Narsaiah K, Thakur B. Functional properties of makhana (Euryale ferox) based value-added products: a review of recent developments. Journal of Food Science and Technology. 2023;60(8):2063–75.

62. Sucharitha S. Processing and value addition to jackfruit (Artocarpus heterophyllus) seed [thesis]. Andhra Pradesh: Acharya N.G. Ranga Agricultural University; 2018.

63. Anaya-Esparza LM, Montalvo-Gonzalez E, Gonzalez-Silva N, Ojeda-Ramirez D, Escalona-Buendia HB, Yahia EM, et al. Effect of

ultrasound treatment on the nutritional, physicochemical and microbiological properties of jackfruit-based beverage. Food Science and Technology International. 2018;24(7):588–98.

64. Dhani S, Hamid, Ngobese NZ, Sharma S, Jaiswal AK. A comprehensive review on nutritional composition, health benefits, and industrial applications of jackfruit seeds. Journal of Agriculture and Food Research. 2025;19:101692.

65. Satheeshan KV, Sucharitha S, Lavanya P, Jayarajan V. Development and quality evaluation of value-added products from jackfruit seed flour. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):2406–10.

66. Dhani S, Hamid, Ngobese NZ, Sharma S, Jaiswal AK. Jackfruit seeds: nutritional composition, health benefits, and food industry applications. Journal of Agriculture and Food Research. 2025; 19:101692.

67. Royees A, Pandey A. Jackfruit seeds: nutritional composition, bioactive compounds, anti-nutritional factors, and applications. Journal of Food Science. 2022;87(10):4204–20.

68. Meira RMF, Lima AMV, Galvao MAM, et al. Prebiotic potential of jackfruit seed polysaccharides in modulating murine gut microbiota. LWT – Food Science and Technology. 2023; 182:114882.

69. Hossain MA, Dewan MF, Billah MT, Ahiduzzaman M, Haque MM, Haque MA. Jackfruit seed as a natural source for protein and mineral enrichment of yogurt. Journal of Food Processing and Preservation. 2023; 2023:9899564.

70. Lumbantobing E, Tanardi S, Putra ABN. Development of vegan ice cream from jackfruit seed-based milk. In: Proceedings of the International Conference on Food Science. Scitepress; 2019.

71. Kundalia A, Mishra A, Rani C, Gupta D, Gautam K, Sushma KM, et al. Nutritional profile, functional characteristics, health benefits,

and potential application of edible gum (Gond). In: Nutritional Science and Technology: Concept to Application. 2023 Sep 14:83–102.

72. Pansare K, Sonawane G, Patil C, Sonawane D, Mahajan S, Somavanshi D, et al. Gond Katira: a natural remedy for summer heat and hydration. Research Journal of Pharmacology and Pharmacodynamics. 2025 May 16;17(2):95–101.

73. Singh M, Raorane CJ, Alka, Shastri D, Raj V, Kim SC, et al. Recent progress on modified gum katira polysaccharides and their various potential applications. Polymers. 2022 Sep 2;14(17):3648.

74. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014 Oct 9;514(7521):181–6.

75. Wazir M, Verma H, Singh J, Singh P, Passey S. The battle of natural sweeteners: a comprehensive guide to monk fruit and stevia. Current Research in Nutrition and Food Science Journal. 2025 Mar 25;13(1):24–45.

76. Kahler E. The effect of natural alternative sweeteners lucuma, yacon, and monk fruit on the growth of probiotic lactic acid bacteria [dissertation]. Dundee: Abertay University; 2020 Dec.

77. Ban Q, Liu Z, Yu C, Sun X, Jiang Y, Cheng J, et al. Physiochemical, rheological, microstructural, and antioxidant properties of yogurt using monk fruit extract as a sweetener. Journal of Dairy Science. 2020 Nov 1;103(11):10006–14.

78. Ruiz-Ojeda FJ, Plaza-Diaz J, Saez-Lara MJ, Gil A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. Advances in Nutrition. 2019;10(Suppl 1):S31–48.

79. Devalekar SK, Udachan IS. Process optimization of vegan prebiotic ice cream by response surface methodology. Discover Food. 2025 Jan 23;5(1):13. doi: 10.1007/s44187-025-00282-0

80. Kowalczyk M, Znamirowska-Piotrowska A, Buniowska-Olejnik M, Pawlos M. Sheep milk symbiotic ice cream: effect of inulin and apple fiber on the survival of five probiotic bacterial strains during simulated in vitro digestion conditions. Nutrients. 2022 Oct 23;14(21):4454. doi: 10.3390/nu14214454

81. Narala VR, Orlovs I, Jugbarde MA, Masin M. Inulin as a fat replacer in pea protein vegan ice cream and its influence on textural properties and sensory attributes. Applied Food Research. 2022 Jun 1;2(1):100066.

82. Narala VR, Jugbarde MA, Orlovs I, Masin M. Inulin as a prebiotic for the growth of vegan yoghurt culture in pea protein-based vegan yoghurt-ice cream, while improving the textural properties. Applied Food Research. 2022 Dec 1;2(2):100136.

83. Lumbantobing E, Tanardi S, Putra ABN. Development of vegan ice cream from jackfruit (Artocarpus heterophyllus) seed-based milk. In: Proceedings of the 16th ASEAN Food Conference. Scitepress; 2019. p. 66–71. doi: 10.5220/0009983700660071

84. Aydemir LY, Demir H, Erbay Z, Kılıçarslan E, Salum P, Ozdemir MB. Production of vegan ice cream: enrichment with fermented hazelnut cake. Fermentation. 2025 Aug 4;11(8):454. doi: 10.3390/fermentation11080454

85. Fransisca F, Dewi RT, Harimas CC. Development and characterization of new vegan ice cream from tempeh milk enriched with moringa leaf flour. Indonesian Food Science and Technology Journal. 2025 Jul 30;8(2):324–335.

86. Hidas KI, Nyulas-Zeke IC, Szepessy A, Romvari V, Gerhart K, Suranyi J, Laczay P, Darnay L. Physical properties of hemp drink-based ice cream with different plant proteins, guar gum, and microbial transglutaminase. LWT – Food Science and Technology. 2023 Jun 1; 182:114865. doi: 10.1016/j.lwt.2023.114865

87. Wyrwisz J, Pokorski P. The impact of psyllium mucilage as a stabilizer on the physical and sensory properties of vegan yogurt ice cream. Applied Sciences. 2025 Oct 19;15(20):11190. doi: 10.3390/app152011190

88. Velotto S, Parafati L, Ariano A, Palmeri R, Pesce F, Planeta D, Alfeo V, Todaro A. Use of stevia and chia seeds for the formulation of

traditional and vegan artisanal ice cream. International Journal of Gastronomy and Food Science. 2021 Dec 1; 26:100441.

89. Acar EB, Karahan T, Mutlu D, Sağdıç O, Öztürk Hİ. Novel vegan ice cream made from red kidney bean milk with a probiotic: technological and biofunctional characteristics. Journal of Food Science. 2025 Mar;90(3):e70087. doi: 10.1111/1750-3841.70087

90. Caponio GR, De Angelis D, Mansueto L, Vacca M, Silletti R, De Angelis M, Difonzo G. Development of plant-based functional ice cream enriched with oat okara: a focus on nutritional, rheological, and sensory properties. LWT – Food Science and Technology. 2025. doi: 10.1016/j.lwt.2025.011740

91. Jan N, Hussain SZ, Naseer B, Bhat TA. Amaranth and quinoa as potential nutraceuticals: a review of anti-nutritional factors, health benefits and their applications in food, medicinal and cosmetic sectors. Food Chemistry Advances. 2023. doi: 10.1016/j.focha.2023.100470

92. Kiełczewska K, Smoczyński M, Gutkowska M. The use of high-protein preparations in ice cream production. Foods. 2025 Jan 21;14(3):345. doi: 10.3390/foods14030345

93. Cruz C, Martinez-Monteagudo SI. Effect of altering the whey protein/casein ratio on quality properties of ice cream. International Journal of Food Properties. 2025;28(1). doi: 10.1080/10942912.2025.2459390

94. Bekiroglu H, Goktas H, Karaibrahim A, Bozkurt F, Sagdic O. Optimizing vegan frozen dessert: the impact of xanthan gum and oat-based milk substitute on rheological and sensory properties. Food Chemistry. 2024. doi: 10.1016/j.foodchem.2024.024373

95. Szydłowska A, Zielińska D, Küçükgöz K, Trząskowska M. Factors affecting the quality of probiotic plant-based frozen desserts — the authors' own experiments in the context of the literature. Fermentation. 2024 May 30;10(6):291. doi: 10.3390/fermentation10060291

96. Limjaroen T, Kijroongrojana K. Yogurt ice cream with Bifidobacterium longum subsp. longum TISTR 2195: effects of fat content and prebiotics on physicochemical and rheological properties, ice cream microstructure, and probiotic survival.

International Journal of Food Science. 2024; 2024:641–656. doi: 10.1080/10942912.2024.2344565

97. Gorman M, Tobin JT, O'Sullivan MG, Kerry JP, McSweeney PLH. Sensory perception of ice cream and plant-based alternatives evaluated blinded and with ingredient lists. Applied Food Research. 2023;3(2):100309. doi: 10.1016/j.afres.2023.100309

98. Abou Ayana IA, El-Aidie SAM, Hassan ZMR. Biofunctional ice cream from novelty prepared oat milk for enhancing nutritional profile and supporting Lactobacillus acidophilus during frozen storage. Industrial Crops and Products. 2025. doi: 10.1016/j.indcrop.2025.008600

99. Hejazi SN, Orsat V. Malting process optimization for antinutritional factor reduction in amaranth grain. Journal of Food Quality. 2016;39(5):557–67.

100. Satheeshan KV, Sucharitha S, Lavanya P, Jayarajan V. Development and quality evaluation of value-added products from jackfruit seed flour. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):2406–10.

101. Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual. San Francisco: Pearson/Benjamin Cummings; 2005.

102. AOAC INTERNATIONAL. Official Methods of Analysis of AOAC INTERNATIONAL. 22nd ed. Oxford University Press; 2023.

103. Food Safety and Standards Authority of India (FSSAI). Food Safety and Standards (Labelling and Display) Regulations, 2020. New Delhi: FSSAI; 2020. Available from: https://fssai.gov.in

104. Indian Council of Medical Research (ICMR). Nutrient Requirements and Recommended Dietary Allowances for Indians. New Delhi: ICMR; 2020.

105. Malik MA, Mushtaq BS. The battle of natural sweeteners: a comprehensive guide to monk fruit and stevia. Current Research in Nutrition and Food Science. 2025;13(1). doi:10.12944/CRNFSJ.13.1

106. Singh M, Raorane CJ, Alka, Shastri D, Raj V, Kim SC, Mishra V. Recent progress on modified gum katira polysaccharides and their various potential applications. Polymers (Basel). 2022;14(17):3648. doi:10.3390/polym14173648

107. Vaishnavi S. Millet-based synbiotic drink: a plant-based alternative to dairy probiotics [dissertation]. Jaipur: NIMS University; 2025.

108. Niness KR, Kelly GS. Inulin and oligofructose: what are they? J Nutr. 1999;129(7 Suppl):1402S–6S. doi:10.1093/jn/129.7.1402S

109. ICMR-NIN. Indian Food Composition Tables (IFCT) 2017. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research; 2017.

110. USDA FDC. United States Department of Agriculture, Food Data Central (FDC). 2024. Available from: https://fdc.nal.usda.gov

111. Ng CKZ, Leng WQ, Lim CH, Du J. Physicochemical property characterization, amino acid profiling, and sensory evaluation of plant-based ice cream incorporated with soy, pea, and milk proteins. J Dairy Sci. 2024;107(12):10268–79. doi:10.3168/jds.2024-25008

Appendix 1

Threshold test

Sensitivity- Threshold test

Name_____

Date: _____

You are provided with a series of containers having solutions with increasing concentration of one of the taste qualities (sweet, salty). Please start with Sr. No. 1 and continue with the rest. The samples are not allowed to be retested. Please describe the taste or give intensity scores using the scoring pattern shown separately here below.

Intensity Score

SET NO.	**Description of the taste and feeling factor**
A	----
B	----
C	----

Scale:

While awarding the intensity scores, take the following basis into account,

O-None or taste of pure water

? -Different from water but taste quality not identifiable

X-Threshold very weak (Taste identifiable)

1- Weak taste

2-Medium

3-Strong

4- Very strong

5. Extremely strong

_____

Signature of Judge

Appendix 2

Evaluation Card for Hedonic Rating Scale

Name _____ Date _____

Product _____

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describe your feelings about the sample. Please give a reason for this attitude. Remember you are the only who can tell what you like. an honest expression of your personal feeling will help us.

Sample code	**Colour**	**Taste**	**After taste**	**Texture**	**Overall Acceptability**

Like Extremely - 9

Like Very Much - 8

Like Moderately - 7

Like Slightly - 6

Neither Like nor Dislike - 5

Dislike Slightly - 4

Dislike Moderately - 3

Dislike Very Much - 2

Dislike Extremely - 1

_____

Signature of Judge

Appendix 3

PARTICIPANT CONSENT FORM

I voluntarily agree to participate in the study title “Formulation of a Prebiotic Plant-Based High-Protein Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum” conducted by Drishti lalwani at Nims university, Jaipur, Rajasthan, India.

I understand that my participation involves:

- Tasting a sample of vegan symbiotic ice cream.
- Providing my opinion on sensory attributes such as **taste, appearance, aroma, texture, and overall acceptability** using a structured questionnaire.
- The duration of participation will be approximately **10–15 minutes**.

I acknowledge that
Participation is completely voluntary.
I may refuse to participate or withdraw at any time without penalty.
My decision will not affect the product.

I understand the nature of the study and procedures involved. I agree to participate voluntarily.

Mobile Number –

Address –

Signature of the participant
Signature of the investigator
Place:
Place:
Date:
Date:

Appendix 4

Seed article

Received: 5 November 2024 | Revised: 24 January 2025 | Accepted: 6 February 2025

DOI: 10.1111/1750-3841.70087

Journal of Food Science WILEY

ORIGINAL ARTICLE

Integrated Food Science

Novel vegan ice cream made from red kidney bean milk with a probiotic: Technological and biofunctional characteristics

Emine Beyda Acar[1,2] | **Talha Karahan**[3] | **Dora Mutlu**[4] | **Osman Sağdıç**[4] | **Hale İnci Öztürk**[4]

[1]Department of Food Engineering, Konya Food and Agriculture University, Konya, Turkey

[2]Konya Sugar Industry and Trade Inc., Snack Products Factory, Konya, Turkey

[3]Department of Molecular Biology and Genetic, Konya Food and Agriculture University, Konya, Turkey

[4]Department of Food Engineering, Yıldız Technical University, Istanbul, Turkey

Correspondence
Hale İnci Öztürk, Department of Food Engineering, Yıldız Technical University, Istanbul, Turkey. Email: inci.ozturk@yildiz.edu.tr

Abstract

This study aimed to develop functional vegan ice creams (ICs) using red kidney bean milk (RKBM), with (RP) and without (R) probiotics (*Bacillus coagulans* ATCC 7050), and to compare their physicochemical, functional, and sensory properties with dairy counterparts. The IC samples had a dry matter content of 33.80%–35.54% and a protein content of 2.05%–2.20%. Although the brightness of the vegan samples was lower compared to dairy samples, the vegan samples were characterized by more reddish and yellowish hues. The vegan samples provided higher overrun values (36.00%–37.50%) compared to the dairy ones. Besides, the vegan samples exhibited higher antioxidant activity (5224–6148 μM Trolox equivalent antioxidant capacity/g) and total phenolic content (TPC) (72.98–184.75 mg gallic acid equivalent/100 g). The processes of ripening, fermentation, and freezing led to an increase in TPC values of all samples. The highest probiotic viability (7.52 $\log_{10}$CFU/g) was observed in the RP mix, but a decrease in probiotic cell count occurred after freezing. The vegan ICs received lower sensory scores than the dairy samples. Despite lower sensory scores, RKBM-based ICs demonstrated suitable functional and technological properties, suggesting that RKBM has significant potential for use in functional vegan food products. Further studies are needed to improve sensory acceptability.

KEYWORDS

functional food, *Phaseolus vulgaris*, phenolic content, plant milk, spore former probiotic

1 | INTRODUCTION

In recent years, the sales of ice cream (IC) and frozen desserts have seen a slight increase, yet the category is evolving to feature more functional, non-dairy, and "better-for-you" options (Mintel, 2019). The market for dairy-free frozen desserts has notably expanded, with traditional IC brands now offering plant-based alternatives. Consumers tend to choose non-dairy ICs because of the perceived health advantages of plant-based diets, as well as concerns about animal welfare and the environmental impact of dairy production (McCarthy et al., 2017).

These developments have played a role in the growth of the market for creative and health-conscious prod-

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Journal of Food Science* published by Wiley Periodicals LLC on behalf of Institute of Food Technologists.